PB81-178261

Development of Bioassay Procedures for Defining Pollution of Harbor Sediments. Part I

Wisconsin Univ.
Superior

Prepared for

Environmental Research Lab.
Duluth, MN

Mar 81

U.S. Department of Commerce
National Technical Information Service
NTIS

PB81-178261

EPA 600/3-81-025
March 1981

FINAL REPORT

DEVELOPMENT OF BIOASSAY PROCEDURES FOR DEFINING POLLUTION OF HARBOR SEDIMENTS

PART I.

by

DONALD A. BAHNICK

WILLIAM A. SWENSON

THOMAS P. MARKEE

DANIEL J. CALL

CRAIG A. ANDERSON

R. TED MORRIS

Center for Lake Superior Environmental Studies
University of Wisconsin, Superior, Wisconsin

PROJECT NUMBER: R804918-01
PROJECT OFFICER: RICHARD ANDERSON
ENVIRONMENTAL RESEARCH LABORATORY
DULUTH, MINNESOTA

PRODUCT OF:
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO.
EPA-600/3-81-025

2.

3. RECIPIENT'S ACCESSION NO.
PB81 17826 1

4. TITLE AND SUBTITLE
Development of Bioassay Procedures for Defining Pollution of Harbor Sediments Part I

5. REPORT DATE
MARCH 1981 ISSUING DATE.

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
Donald A. Bahnick, William A. Swenson, Thomas P. Markee, Daniel J. Call, Craig A. Anderson, and R. Ted Morris

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Center for Lake Superior Environmental Studies
University of Wisconsin-Superior
Superior, Wisconsin

10. PROGRAM ELEMENT NO.

11. CONTRACT/GRANT NO.
R804918-01

12. SPONSORING AGENCY NAME AND ADDRESS
U.S. Environmental Protection Agency
Environmental Research Laboratory-Duluth
6201 Congdon Boulevard
Duluth, Minnesota 55804

13. TYPE OF REPORT AND PERIOD COVERED

14. SPONSORING AGENCY CODE
EPA-600/03

15. SUPPLEMENTARY NOTES

16. ABSTRACT

This research investigates bioassay methods which may be useful in assessing the degree of pollution of harbor sediments. Procedures studied include 96 hr toxicity tests employing Hexagenia limbata, Daphnia magna and Pontoporeia affinis as biological probes, monitoring cough frequencies of bluegill sunfish (Lepomis macrochirus) in interstitial water derived from sediments, chemical analyses of sediment-water systems, and chemical analysis of chironomids and Hexagenia limbata exposed to the sediments. Additional experiments involved investigation of the degree of removal of chemical constituents from sediments due to extraction with Lake Superior water and the use of reverse phase liquid chromatography in detecting the presence of chemical compounds with high bioaccumulation potential in the sediments.

A general toxicity index was prepared from the chemical data which indicated that animal survival in the 96 hr acute toxicity tests was generally lower using sediment systems from the more industrialized areas of the harbor.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT
RELEASE TO PUBLIC

19. SECURITY CLASS *(This Report)*
UNCLASSIFIED

20. SECURITY CLASS *(This page)*
UNCLASSIFIED

21. NO. OF PAGES

22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

CONTENTS

Section 1 - Introduction ii

Section 2 - Conclusions. iii

Section 3 - Recommendations. v

Section 4 - Overview of the Study. 1
  Background and Purpose. 1
  Biological Tests to Screen Sediments. 3
    Daphnia Bioassays. 3
    Hexagenia Bioassays. 4
    Pontoperia Bioassays 5
    Fish Behavior Bioassay 5
  Sediment Employed and Chemical Characterization 6
  Uptake of Chemicals by Chironomids and Hexagenia. 7
  Bioaccumulation Determinations Using High Liquid Pressure Chromatography 7
  Chemical Correlations to Bioassays Using Biological Probes. 8
  Development of Harbor Sediment Sampler. 8

Section 5 - Study Area 10
  General Harbor Description. 10
  History of Pollution. 12
  Physical Nature of the Sediments. 13
  Chemical Nature of the Sediments. 14
  Aquatic Life. 16

Section 6 - Project Field Sampling 18
  Sampling Sites. 18
  Sediment, Water and Chironomids Sampling. 19
  Hexagenia Limbata 21
  Pontoporeia Affinis 21
  Lepomis Macrochirus 23

Section 7 - Chemical Characterization of Samples and Water - Sediment System Preparation 24
  Sediment-Water Systems Utilized 24
  Treatment of Core Samples 24
    Background 24
    Procedure. 25
  Sediment Analysis 27
    General Physical and Chemical Sediment Tests 28
    Preparation of Sediment Extracts for Metal Analysis. 28
      Exchangeable Phase Metals 32
      Easily Reducible Phase Metals 32
      Organic and Sulfide Phase Metals. 33
      Moderately Reducible Phase Metals 33
      Residual Phase Metals 34
    Metal Analysis Procedures. 35
    Metal Analysis Results 35
    Analysis for Total Metals in Sediments 41
    Pesticide and PCB Analysis 41

        - Sediment Extraction Procedure 44
        - Extract Clean-Up Procedure 44
        - Separation of PCBs and Pesticides 45
        - Gas Chromatographic Analysis 45
    - Chlorinated Phenol Analysis 46
        - Analysis Procedure 46
    - PAH Analysis 48
        - Analysis Procedure 49
        - Analytical Results 50
    - Discussion of Sediment Results 53
        - Metals in the Sediments 54
        - Trace Organics in the Sediments 55
  - Overlying Water 56
  - Interstitial Water 59
  - Elutriate Water 63
    - Preparation of Liquid Phase (Elutriate Water) and Particulate Phase 64
    - Liquid Phase (Elutriate Water) and Particulate Phase Tests 65
    - Survey of Chemical Properties of Elutriate Water 65
  - Generated Pore Water 70
    - Generated Pore Water Production Procedure 71
    - Generated Pore Water Results 72
    - Discussions of Results 74

- Section 8 - Bioassay Tests 83
  - General Bioassay Procedures 83
    - Light and Temperature Control 83
    - Daphnia Culturing Techniques 83
    - Acclimation and Handling of Test Organisms 84
  - Oversediment Bioassay 86
    - Methods 86
      - Hexagenia-Daphnia Bioassay Methods 86
      - Pontoporeia affinis Bioassay Methods 90
    - Results 91
      - Hexagenia Bioassays 91
      - Daphnia Bioassays 94
      - Pontoporeia Bioassays 98
  - Elutriate, Interstitial and Pore Water Bioassays 100
    - Methods 100
      - Daphnia Bioassay 100
      - Fish Bioassays 101
    - Results 106
      - Daphnia Bioassays 106
      - Fish Bioassays 109
  - Comparisons of Results to Other Sediment Bioassay Studies 110

- Section 9 - Bioaccumulation Potential of Sediment Associated Chemicals 114
  - Chemical Analysis of Hexagenia and Chironomids 114
    - General Methods 114
    - Results 115
      - Bioaccumulation of PCBs 116
      - Bioaccumulation of Pesticides 116
      - Bioaccumulation of PAH Compounds 118
      - Bioaccumulation of Metals 121
    - Related Studies 122
  - Sampling Screening for Organic Chemicals Using HPLC 123
    - Methods and Samples 124
    - Results 125
    - Discussion 128

Section 10 - Relationships Between Chemical Characteristics of the Sediment Systems and Bioassay Toxicity 130
  Site Location and Toxicity 130
  General Site Chemistry and Toxicity. 131
  Specific Chemicals and Toxicity. 133

References. 139
Appendices. 145

SECTION 1

INTRODUCTION

This study was undertaken to evaluate bioassay techniques which might be applicable to assessing potential harmful effects resulting from the dredging and disposal of harbor sediments. Sediments from the Duluth, Minnesota and Superior, Wisconsin harbor area were used in preparing systems containing water overlying a sediment substrate, containing interstitial water, or containing elutriate water. Acute 96 hour toxicity tests were carried out by exposing Hexagenia limbata, Daphnia magna and Pontoporeia affinus to certain of these systems. Bluegill sunfish (Lepomis macrochirus) were monitored for cough frequencies and breathing patterns in sediment interstitial water mixed with Lake Superior water.

Sediment quality was chemically evaluated by extensive analysis of the sediments and their interstitial waters for a variety of chemical parameters. The chemical testing included determinations of metals, certain inorganic nonmetallic substances, particle size, pH, Eh and trace organics (PCBs, pesticides, and PAH compounds). The results of the chemical analyses were compared to the biological toxicity tests when possible.

Chironomids collected from various harbor sites and Hexagenia limbata exposed for 96 hours to harbor sediments were analyzed to determine accumulation of specific organic compounds (PCBs, PAH and pesticides) and some heavy metals.

Harbor sediments, harbor chironomids and the sediment exposed Hexagenia limbata were extracted into organic solvents. The lower molecular weight organic components in the extracts were separated by reverse phase high pressure liquid chromatography. This chromatographic study was investigated for its possible use in evaluating the presence of organic compounds having high lipid bioaccumulation potential.

Part II of this project evaluated a method of assessing sediment quality by exposing specific enzymes to extracts from the sediments and monitoring changes in enzyme activity.

SECTION 2

CONCLUSIONS

On the basis of one or more chemical parameters, most of the sediment samples used in the bioassay tests would be classified as polluted according to the currently used sediment evaluation criteria given in Appendix D. Ranking the sediments according to their concentrations of a large number of metals, inorganic non-metals and organic chemical parameters indicated that sites located in the Superior harbor (near the Superior entry to Lake Superior) and a Lake Superior site were less polluted than sites located near the more industrialized zones in the harbor.

Chemical analysis for heavy metals in the sediments revealed that the residual phase of the sediments contained the highest concentrations of most of them. However measurable amounts (>1 mg/kg) of arsenic, cobalt, copper, nickel and zinc were found in the organic and sulfide sediment phases for nearly all the samples and selenium and cadmium were found in these phases for some of the samples.

PCB concentrations in the sediments ranged from 0.3 to 2.1 mg/kg based on a dry sediment basis. These values are not high compared to sediments from other harbor areas. In addition to PCBs, low levels of polycyclic aromatic hydrocarbons were found in two of the harbor sediments and low levels (1 to 15 μg/kg) of pentachlorophenol were detected.

Studies on determining the amounts of chemical species which would be released upon flushing the sediments with Lake Superior water showed that only about one per cent or less of most of the chemicals (COD, Fe, Mn, Ni, Pb, Cu, Zn, Hg) were removed from the sediments by water extraction. These results indicated that the measured chemical species were not readily available to water except when associated with particulates.

Chemical analysis of sediment interstitial water showed that many of the chemical species were probably associated with very fine (possibly colloidal) particles in the water. The concentrations of many of the metals were much lower in filtered water samples compared to non-filtered samples.

The concentrations of chemicals found in liquid phase elutriate water did not change greatly when prepared from an exposed sediment or from sediment unexposed to air.

Acute toxicity tests using sediment-water systems resulted in generally low toxicity to *Hexagenia limbata*, *Daphnia magna* and *Pontoporeia affinis*. The high survival of tests animals indicated low levels of available toxicants in the sediments. In 96 hour bioassays, survival was found to be significantly lower for test sites compared to controls in only a few tests. The results demonstrated that the animals could be successfully maintained in the complex test systems and the sediment-water systems caused low acute toxicity. This low toxicity in combination with low precision between replicates generally resulted in finding no significant differences in animal survival between test and control. Among the test species employed, *Daphnia magna* appeared to be the most sensitive to toxicants.

The average cough frequencies of bluegills in dechlorinated city water and in interstitial water from the sampling site sediments mixed with Lake Superior water were generally similar. Cough frequencies during the first 22 to 26 hours of fish exposure were found to be elevated above the frequencies observed for the control for three of the six sites studied. Bluegill opercular activity was nearly continuous in dechlorinated city water in contrast to broken patterns of activity observed in interstitial water - Lake Superior water mixtures formed from sediments obtained from five of the six sampling sites.

Although survival of test organisms was generally high during the 96 hour toxicity tests, some correlations of survival of *Daphnia magna* in water overlying sediments (1977 tests) with chemical parameters were found. Many of these correlations involved the concentrations of metals in the sediment, in interstitial water removed from the sediment or in elutriate water formed by mixing sediment with Lake Superior water.

Some correlations were found between *Daphnia magna* survival in elutriate water - Lake Superior water mixtures and individual chemical parameters. Correlations were found between iron concentrations in interstitial water removed from the sediments and *Daphnia* survival.

A general index of toxic effects, developed by considering the relative percentage of low survival for the various acute toxicity tests, showed that survival was generally lower in test systems derived from sediments in the industrialized areas of the harbor compared to the less developed areas and the Lake Superior site. Comparing the percent low survival values for the sites to rankings based on sediment and interstitial water chemical results indicated a positive correlation to the rankings based on sediment chemistry ($r = 0.80$, $P > 0.1$). A lower correlation coefficient was found between rankings based on interstitial water chemistry and the percent low survival values. These results indicate that the combined chemical tests for the sediments were a fair indicator of general toxicity.

Evidence was found that chironomids dwelling in harbor sediments had accumulated PCBs and p,p'-DDE. Similar results were found for *Hexagenia limbata* exposed to harbor sediments for 96 hours.

Chromatograms of organic extracts from sediments, chironomids and *Hexagenia limbata*, using reverse phase high pressure liquid chromatography, showed the presence of organic compounds with bioaccumulation potential. This method of screening samples for the amounts of bioaccumulated organic compounds is potentially useful.

SECTION 3

RECOMMENDATIONS

The use of Daphnia magna, Hexagenia limbata or Pontoporeia affinis as test organisms for potential toxic effects of sediments should be considered further. Of these species, Daphnia magna appears to be most suited as a test organism due to ease of culturing, sensitivity and the large amount of information available on the response of Daphnia to specific chemicals. Further tests would be useful employing sediment samples having greater variation in chemical quality. Recognizing that sediments used in this study contained large quantities of potentially toxic heavy metals in unavailable or nontoxic forms, it is important to develop better understanding of the conditions which would result in transformation to available forms and the effects that such transformations would have. Comparisons of toxicity results to other recently developed toxicant screening techniques such as algal or luminescent bacteria assays are recommended.

It was necessary to design the tests following approved criteria for ecological evaluation of dredging and dredge spoil disposal in marine systems. Following these criteria, controls for the bioassay tests were derived from sediments from Lake Superior and a relatively undisturbed area of the harbor. Because these sediments contained varying quantities of many toxic substances, their use negated accurate determination of the sensitivity of the bioassay procedures. It is therefore recommended that future studies aimed at identifying screening procedures incorporate more effective controls.

Based on our observations, bluegill cough frequencies are difficult to interpret and their usefulness in determining differences in sediment quality was limited due to observed similarities in results for the various sites. The data suggests that extensive experience in conducting fish cough response tests is necessary to interpret the results, and therefore the technique has limited applications as a general screening procedure.

Further analysis of bioassay results and chemical characteristics of the sediments is desirable. If correlations hold for a wide variety of sediments, additional understanding of the causes of the observed toxicity results may be obtained.

Screening of sediment extracts or extracts of animals exposed to sediments using reverse phase high pressure liquid chromatography should be further investigated. In particular, the eluent fractions containing organic compounds with high lipid solubility should be studied for methods to quantitate and perhaps identify highly bioaccumuable compounds.

SECTION 4

OVERVIEW OF THE STUDY

BACKGROUND AND PURPOSE

The need for maintaining vessel accessibility to our nation's waterways necessitates a continuous dredging program by the Corps of Engineers. The enormity of this program is demonstrated by the 290 million cubic meters of sediment dredged annually in the United States. About 65% of this dredged material is deposited in streams, lakes and coastal waters (Boyd *et al.*, 1972). The prediction of ecological perturbations due to dredging activities is difficult. The need for a reliable evaluation procedure to identify the potential effects of chemical contaminants in dredged or fill materials on water quality and the aquatic community is recognized.

Section 103 of Public Law 92-532 (Marine Protection, Research and Sanctuaries Act of 1972) requires the evaluation of dredged material proposed to be discharged into ocean waters. Guidelines for this evaluation have been presented in the *Federal Register*, Vol. 42, No. 7, Tuesday, 11 January 1977. An implementation manual for this evaluation has been prepared (EPA/Corps of Engineers, 1977) which incorporates toxicity bioassays, analysis of chemical constituents and bioaccumulation tests in the procedure. The manual recognizes the dynamic status of ecological evaluation methods and the need to develop new procedures. The evaluative methods included in this manual, in conjunction with on-going and recently completed research studies, will probably form the framework for an evaluative manual covering the effects of fresh water dredging activities on ecosystems.

The current criteria for the evaluation of the quality of Great Lakes harbor sediments is based on EPA guidelines (EPA, 1977). These criteria are based on levels of chemical parameters in sediments (principally total volatile solids, chemical oxygen demand (COD), total Kjeldahl nitrogen, oil and grease, mercury, lead and zinc although ammonia, cyanide, phosphorus and a number of other metals may be considered), field observations and benthos. However, projection of bulk sediment chemical analyses to potential adverse ecological effects due to dredging activities is difficult. The criteria do not identify the fractions of sediment which are potentially toxic and biologically available. The problem of bioaccumulation of chemicals in flora and fauna resulting in magnification in aquatic food chains is not adequately addressed.

In recognition of the need for a comprehensive program to assess and predict the environmental aspects of dredging and dredged material disposal operations, the Corps of Engineers has conducted a five year Dredge Material Research Program (DMRP) through its Engineers Waterways Experimental Station (WES) aimed primarily at ocean disposal of dredged spoils. This program addresses a wide variety of environmental considerations due to dredging including the mechanism and the magnitude of chemical constituent release from dredge material and subsequent effects. Due to the tremendous quantities of sediment dredged annually and the extreme complexity in assessing environmental impacts, it is essential to explore a variety of approaches aimed at assessing and predicting potential effects.

This research attempts to develop procedures designed to assess potential harmful effects of harbor sediments subject to dredging. In pursuing this objective, the applicability, ease of duplication and cost effectiveness of the methods was taken into consideration. Short term bioassay tests using fish, benthos and plankton were studied for their suitability in screening sediment quality. These tests utilized sediment from the Duluth-Superior harbor and Lake Superior and the freshwater species _Hexagenia limbata_ (burrowing fauna), _Daphnia magna_ (planktonic fauna) and _Pontoporeia affinis_ (burrowing fauna) in 96 hr toxicity determinations. Bluegill sunfish (*Lepomis macrochirus*) served as the biological probe in cough frequency and breathing pattern measurements. Information on the usefulness of changes in the activity of specific enzymes as a tool to monitor sediment quality was developed. The sediment and water systems used in these tests were chemically characterized for a large number of parameters. Bioaccumulation of PCBs and p,p'-DDE and ten metals were studied in Chironomid larvae obtained from harbor sediment sites and *Hexagenia limbata* exposed to sediment in the 96 hr tests. The use of high pressure liquid chromatography to screen sediments for bioaccumuable organic material was also investigated. Attempts were made to correlate chemical properties of the sediment and water systems to the tests employing the biological probes.

The results of this project should aid in identifying methods useful in assessing the pollution status of harbor sediments and possible adverse ecological effects associated with dredging and disposal of spoils. The development of meaningful methods is necessary to avoid ecosystem perturbations and, at the same time, minimize the cost and delay factors associated with removing sediments from our waterways and their subsequent deposition.

BIOLOGICAL TESTS TO SCREEN SEDIMENTS

Daphnia Bioassays

Daphnia sp. have been widely used in screening bioassays for a variety of individual toxicants (Biesinger and Christensen, 1972; Anderson, 1944; Macek *et al*., 1976) and complex effluents (Winner, 1976; Arthur *et al*., 1975). *Daphnia* have been shown to exhibit sensitivity to a varity of chemical species. The available data on *Daphnia* responses coupled with their short generation time and ease of culturing suggests that they are a suitable group for use in harbor sediment screening bioassays.

Acute 96 hour toxicity tests using Daphnia magna were conducted in situations causing exposure of these animals to Lake Superior water in contact test sediments. Additional 96 hr tests resulted in determining toxic effects of interstitial water extracted from the sediments, elutriate water prepared from Lake Superior water-sediment suspensions (EPA/Corps of Engineers, 1977; Keeley and Engler, 1974), and Lake Superior water used as a sediment extractant (generated pore water). Elutriate water was prepared by shaking sediment with Lake Superior water, allowing the sediment to settle for one half hour and centrifuging the suspension (elutriate particulate phase) to remove most of the suspended particles. The effect of suspended particles on Daphnia magna survival was studied by exposure of the animals to mixtures of test sediment elutriate particulate phase with Lake Superior water.

Hexagenia Bioassays

Hexagenia sp. possess characteristics highly suitable for use in sediment bioassay tests. The group has a wide distribution and is sensitive to changes in sediment and related water quality (Fremling, 1964; Fremling, 1967). Eriksen (1963) found Hexagenia nymphs to occupy water within 7 m above the sediments. The animals ingest mud, organic detritus, bacteria and algae from above, on or below the sediment surface. Smith and Oseid (1974) found behavioral changes in Hexagenia limbata occurred at below lethal concentrations of hydrogen sulfide. The animals were found to be suitable for bioassay tests with oxygen concentrations down to 2 ppm. Prater and Anderson (1977, 1977a) have utilized this species in acute toxicity tests of sediments. The burrowing behavior of Hexagenia facilitates direct contact with the sediments thus promoting uptake of bioavailable toxicants associated with the particulates and interstitial waters. The relatively large size of the nymphs provides adequate tissue sample for chemical analysis.

Behavior studies and acute 96 hour toxicity tests were carried out using Hexagenia limbata in aquaria containing test sediments and overlying Lake Superior water. The locations of the animals were observed over this period and their survival was noted at the conclusion of the tests.

Pontoporeia Bioassays

Pontoporeia affinis is the predominate member of the macrobenthic fauna in all the Great Lakes. Although a burrowing species, it undergoes vertical migration in the water column, making it a highly versatile sediment quality probe. Changes in activity have been correlated with bioaccumulation of mercury from sediments (Magnuson, et al., 1976). Previous studies demonstrated that P. affinis show sediment behavior which may be related to sediment quality (Marzolf, 1965; Gannon and Beeton, 1969).

Acute 96 hour bioassay tests were conducted involving the exposure of Pontoporeia affinis to elutriate water-Lake Superior water mixtures in systems containing sedimented particulates from the test sites as substrates. The survival of the animals at the conclusion of the tests was recorded.

Fish Behavior Bioassay

Cough response of several species of fish has been used as an effective indicator of acutely toxic concentrations of individual pollutants (Drummond, et al., 1973; 1974) and complex effluents (Walden, et al., 1970). Recent studies of complex effluent screening suggests application of the procedure using small volumes of test water in 24-48 hr fish exposures (Carlson and Drummond, 1978). Due to the sensitivity of the method and its potential low cost, it was tested for its applicability to screening harbor sediment quality.

In these tests, bluegill sunfish (Lepomis macrochirus) were exposed to Lake Superior water and to mixtures of Lake Superior water and interstitial water obtained from harbor sediments for a period of 96 hr. Cough frequency and change in breathing patterns were measured as end points for these tests.

SYSTEMS EMPLOYED AND CHEMICAL CHARACTERIZATION

The various tests using biological probes to monitor sediment quality employed both sediment and liquid phase exposure systems. Chemical and physical analyses of these systems were made for numerous parameters which would characterize the test media and allow chemical correlations to the acute toxicity bioassay results. The bioassay tests employing fish, benthos and plankton as probes were studied during the summer of 1977 and 1978. The chemical characterizations of the test sediments and aqueous phases were more extensive during the summer of 1977 than during the summer of 1978.

The exposure systems consisted of sediment, water overlying the sediment, interstitial water extracted from the sediment under anaerobic conditions, elutriate water prepared from Lake Superior water mixed with sediment which was either exposed to air or kept under nitrogen prior to elutriate formation and Lake Superior water used to extract water solubles or colloids (generated pore water). During 1978, particulate phase elutriate water was also used as an animal exposure system.

The sediments were generally analyzed for the "Jensen Criteria" parameters and additional metal content indicative of the chemical form of the metals and their biological availability. The sediment metal characterizations were made according to the amounts in the interstitial water, exchangeable phase, easily reducible phase, organic and sulfide phase, moderately reducible phase and residual phase metals (Engler, et al., 1974; Chen, et al., 1976). Analyses of sediments for specific organic compounds included determinations of some polychlorinated biphenyls (PCB) and pesticides, some polynuclear aromatic hydrocarbons (PAH) and pentachlorophenol. The sediments (cores) were also analyzed for particle size, pH and Eh.

The water phases were generally analyzed for heavy metals, iron, oxygen content, pH, chemical oxygen demand (COD), NH_3, H_2S, chloride, suspended solids and some trace organics. The waters used in certain of the bioassay studies were analyzed for most of these parameters.

UPTAKE OF CHEMICALS BY CHIRONOMIDS AND HEXAGENIA

Samples of Chironomids were obtained from the sediment sites within the Duluth-Superior Harbor. Hexagenia limbata exposed to test sediments for 96 hr in the acute toxicity studies were collected. These biological samples were analyzed for some specific organic compounds (PCB, PAH, pesticides) and some heavy metals. Determination of the accumulation of these chemicals in the benthic organisms was undertaken to better understand the relationships between sediment concentrations, bioavailability and bioaccumulation potential. Some recent studies have shown that the available form of organic chemicals includes some of the chemical adsorbed to fine particulate matter (Nimmo, et al., 1971; Courtney and Denton, 1976; Zitko, 1974; Nathans and Bechtel, 1976). A number of metals (primarily methylated forms) accumulate in organisms where they may lead to adverse effects on the animal or render it unfit for human consumption (Wood, 1974).

BIOACCUMULATION DETERMINATIONS USING HIGH PRESSURE LIQUID CHROMATOGRAPHY

A measure of the relative tendency of an organic chemical to bioaccumulate in the lipids of animals is given by its n-octanol/water partition coefficient

(P). For example, values of log P have been used to predict the bioconcentration factors of organic chemicals in fish (Neely, *et al*., 1974). Recent studies have shown that log P values for a large number of organic chemicals can be correlated to their retention times on a reverse phase column employing a high pressure liquid chromatograph (HPLC) with methanol-water mixtures as the mobile phase (Veith and Austin, 1976; Veith and Morris, 1978). Since it would be very time consuming and expensive to attempt to identify and quantitate the complex mixture of organic chemicals in sediments, screening of organic extracts by HPLC may prove to be a rapid and inexpensive method to evaluate their potential bioaccumulation hazard.

Organic extracts from harbor sediment sites and a Lake Superior site were injected into a HPLC system and the eluting chemicals were monitored by ultraviolet absorption. Similar HPLC separations of extracts from harbor site Chironomids and *Hexagenia limbata* were carried out. The extracts were qualitatively evaluated for the presence of chemicals with high log P values and a summary of method feasibility has been presented.

CHEMICAL CORRELATIONS TO BIOASSAYS USING BIOLOGICAL PROBES

The results of the bioassays using animal toxicity and fish behavior as end points have been analyzed through regression analyses for correlations with chemical characteristics of the sediments and water systems employed. Chemical parameters in the various water systems were investigated for toxic levels when possible. Speculations on the feasibility of using chemical parameters pertaining to the sediments and water systems to predict adverse ecological effects of dredging have been made.

Sediments from test sites were ranked based on chemical analysis of a large number of parameters pertaining to the sediments or the interstitial water extracted from the sediments. The numerical rankings gave an indication of the relative amounts of the chemical species contained in the sediments or interstitial water. The rankings were tested for correlations to the animal survival results in order to determine whether or not sediment and/or interstitial water chemistry were predictors of toxic effects in the bioassay systems.

SECTION 5

STUDY AREA

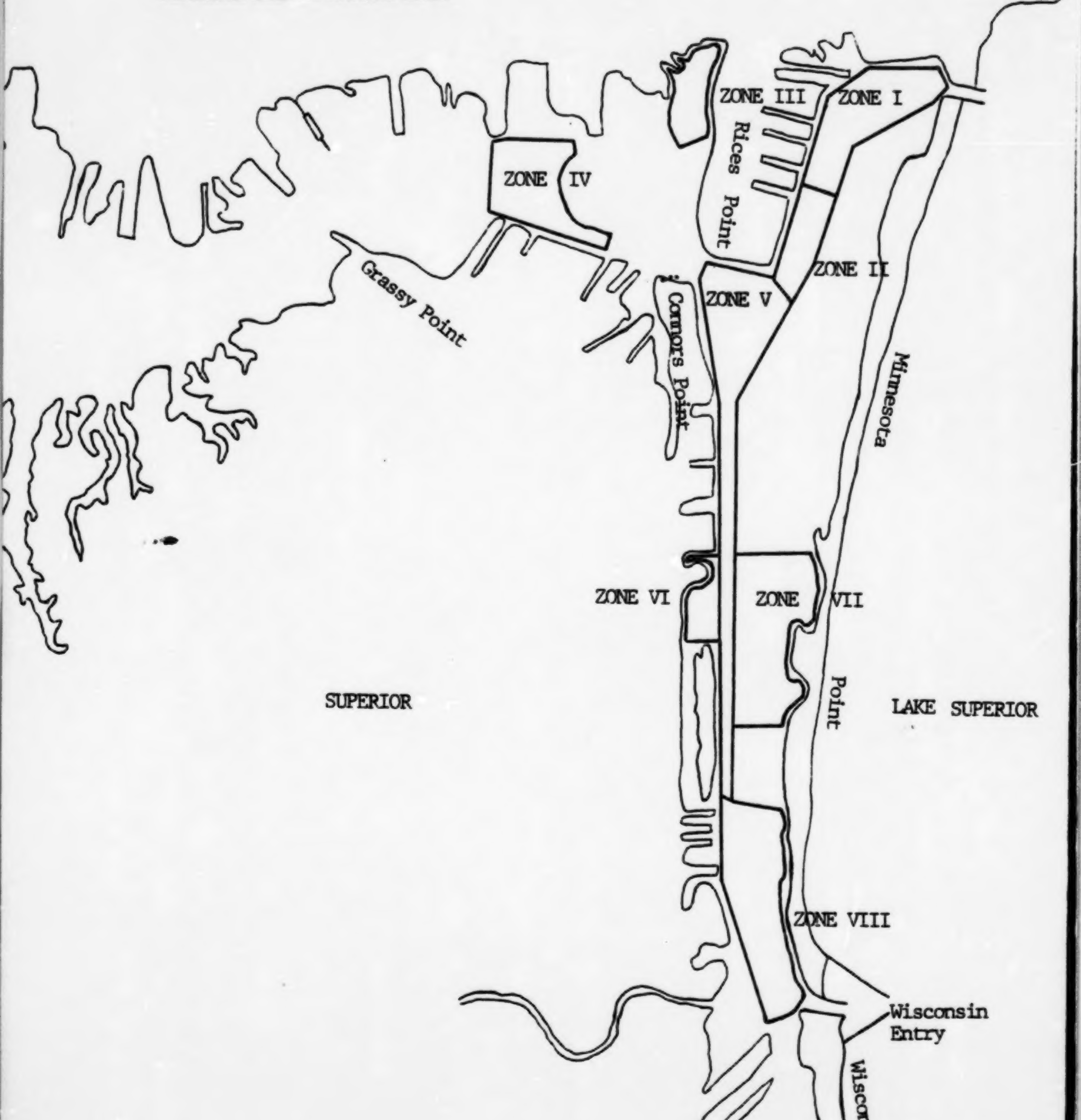
GENERAL HARBOR DESCRIPTION

The Superior-Duluth harbor includes over 11,500 acres of which 3,100 acres is dredged channel, 22-27 feet deep; 650 acres is natural river channel, 6-15 feet deep and; 7,750 acres is breakwater bays and sloughs, less than 6 feet deep (DeVore 1978). The area is characteristic of most estuaries being historically shallow and possessing critical habitat for shore birds, waterfowl and fish in addition to industrial development and shipping.

The estuary is located at the southwest tip of Lake Superior and is separated from the main lake by the largest natural freshwater bay-mouth sand bars in the world; Wisconsin and Minnesota Points (Figure 1). A natural opening through the bars to the lake was maintained in the early years by the currents of the Nemadji and St. Louis Rivers at a location which is now the Wisconsin entry (Figure 1).

The harbor was geologically formed by the combination of declining water levels and a subsequent rise of the north side of the lake basin. Declining water levels resulted in construction of a series of three parallel sets of sand spits (beaches). The last two sets of these bars divide the harbor from the lake (Wisconsin and Minnesota Points) and the outer harbor from the inner harbor (Connors and Rices Points). A third and older set form the base of the Grassy Point and Arrowhead bridges (Figure 1). Subsequent to the water level decline, rebound of the northshore from the weight of glacial ice has resulted in flooding of the St. Louis and Nemadji River mouths and the low lying areas between the sand spits. The effect of this natural change was formuation of these bays which make up the harbor. Superior and Allouez Bays were formed

FIGURE: 1
SUPERIOR-DULUTH HARBOR AREA


ZONE III
ZONE I
ZONE IV
Rices Point
ZONE II
Grassy Point
ZONE V
Connors Point
Minnesota
ZONE VI
ZONE VII
SUPERIOR
Point
LAKE SUPERIOR
ZONE VIII
Wisconsin Entry
Wisconsin Point

by inundation of the low land between the two most recently formed sets of sand bars. St. Louis Bay which makes up the inner harbor was formed by flooding of the St. Louis River mouth and low lands between the inner two sets of sand bars.

The partial separation of Allouez and Superior Bays (Section 6, Figure 2) undoubtly results from sediments deposited by the Nemadji River. The Nemadji flows through highly erodable unconsolidated red-clay sediments which were deposited during high water stages of the lake. The red turbid water of the Nemadji deposits much of its sediment load in the harbor, the natural entry and the lake proper.

The red water and sediment deposited in the harbor made navigation difficult during the early settlement years. To solve this problem piers were constructed in the natural entry to narrow and deepen the channel and dredging was initiated in the 1860's. Since that time, over 55 million cubic yards of sediment has been dredged to form the extensive network of channels, slips, docks and a second entry; the Minnesota entry located on the northwest end of the harbor. In addition to being responsible for development of deep water areas, deposition of spoil is responsible for development of all the major islands in the lower harbor (Barkers, Hog and Herders) and the formation of some shoal water areas. Over the years a large percentage of the spoil has been deposited in the open lake (Corps of Engineers 1974).

Establishment of navigation channels was stimulated by mining of Mesabe Range ore deposits, the harvest of native white pine forest stands and wheat farming in the plains states. The improvements in navigation channels resulted in rapid industrial development along the shoreline and the growth of the Superior, Wisconsin-Duluth, Minnesota metropolitan area, the largest on Lake Superior. Iron ore or iron concentrate (taconite) has remained the primary commodity shipped from the port since the early years. Montana coal and grain represent the second and third most important cargos shipped in recent years. Salt, limestone, petroleum products, cement, fish and steel are also shipped through the port. Total shipments during the period from 1967-1972 ranged between 37-43 million tons. Construction of a major coal shipment facility in 1974 has resulted in a substantial increase in shipments of coal.

HISTORY OF POLLUTION

Water quality, which is often considered a modern problem, declined in the Superior-Duluth estuary during the early period of development. Newspaper accounts describing pollution of the St. Louis River indicate sludge mats and methane gas odors occurred in the upper harbor to Cloquet as early as 1914 and resulted from Kraft mill effluents and saw mill wastes. Water quality monitoring was begun by the city of Duluth in 1946 and showed mid-summer dissolved oxygen below the minimum requirements for most fish species during the first year of record. Reduced oxygen undoubtly resulted from the decay of wood fiber mats, municipal sewage (which was not treated by either city prior to 1950) and ship discharges of sewage.

Harbor zones were identified in the 1972-1973 impact assessment program according to forces and sources of contamination which influence them (National Biocentrics Inc. 1973). Zone I near the Duluth, Minnesota entry (Figure 1) is influenced by Lake Superior storm wave activity, vessel movement and storm runoff from the Duluth sewage system (National Biocentrics Inc. 1973). The western edge of this zone and zone II, the Duluth turning basin, are also influenced by grain dust which introduces the potential pesticide contamination and by other industrial developments. Zone III represents a shallow backwater area in the inner harbor previously used for dredged spoil disposal which also received discharges from the Duluth municipal treatment facility.

The St. Louis River represents the dominant force in the inner harbor area represented by Zone IV. In addition to paper mill effluents and sediments carried by the river, grain elevators, coal docks, ore docks, and a Minnesota Power and Light plant are located in the inner harbor and represent potential sources of contaminants. Fly ash deposits have been plowed into the harbor by the power station and represent one of the most obvious potential hazard areas in this zone.

Zones V, VI and VII include a deep water channel area (Zone V) influenced primarily by ship movement; a shallow area (Zone VI) which historically has received storm and direct sanitary sewage discharges from the Superior, Wisconsin treatment plant and; a shallow zone (Zone VII) bordered by a relatively undeveloped shoreline. The area near and including the Superior entry (Zone VIII) is influenced by Lake Superior seiches, major sediment discharges from the Nemadji River and ship movement into ore and ore concentrate loading facilities located in the area.

PHYSICAL NATURE OF THE SEDIMENTS

No quantitive information on physical or chemical quality of harbor sediments was available prior to 1970 except for engineering data obtained from sediment cores taken prior to channel modification work[1] and at construction sites. However, during the period between 1970-1977 extensive sampling was conducted to define the physical-chemical nature of harbor and western Lake Superior sediments. Harbor data were collected by the U.S. Environmental Protection Agency (15 samples, 1970; 6 samples 1973; 33 samples 1975; 6 samples 1976) National Biocentrics Incorporated (16 samples; 1972-1973) the University of Wisconsin-Superior (36 samples, 1973) the University of Wisconsin-Madison (6 samples and the U.S. Army Corps of Engineers (60 samples, 1977). An additional 13 sites were analyzed by the University of Wisconsin-Madison.

---

[1] Corps of Engineers, Boring Logs for the Superior-Duluth harbor 1958-1961.

Sediment borings taken during 1958-1961 by the U.S. Corps of Engineers from the inner harbor and Allouez Bay (areas not previously dredged) show the sediments below the surface to be inorganic silts and clays which are underlaid by silty sands in some areas. These borings and surface samples (power dredge, National Biocentrics Inc., 1973) show the upper layers in the inner harbor and Superior entry area to be composed of poorly sorted mixtures of clay and silt. Sieve analysis of these sediments showed the inner harbor and Superior entry area (which are influenced by the St. Louis and Nemadji Rivers) are comprised of over 45% silt-clay sized particles whereas, sediment from other areas of the harbor seldom contained over 20% silt and clay sized particles.

Comparisons of harbor and lake sediments made by Van Tassel and Moore (1976) showed that harbor sediments in general contained a higher percentage of clay sized particles and were not as well sorted as lake sediments. The lake sediment studies indicate that currents remove finer particles from inshore areas, and transport them to deep water zones. The general pattern results in removal of fine sediments from the Wisconsin inshore area and deposition in deeper offshore Minnesota waters.

CHEMICAL NATURE OF THE SEDIMENTS

Chemical analysis performed during 1970-1973 were conducted to define the quality of harbor sediments with respect to EPA guidelines (EPA criteria as given in Appendix D) for volatile solids, chemical oxygen demand, total Kjeldahl Nitrogen, total phosphorus, oil and grease, mercury, lead, and zinc. In addition to the above, arsenic, cadmium and copper were measured during most investigations. The chemical analysis demonstrated that some samples in every zone exceeded the guidelines for most parameters and that the chemical nature of the sediments was extremely variable within and among harbor zones (National Biocentrics Inc., 1973).

Correlations analysis showed that heavy metal and oil and grease concentrations were directly related to the percentages of clay and silt sized particles in the sediments, supporting the hypothesis that fine particles provide more binding sites and combine with metals and organic compounds in the sediments (National Biocentrics Inc., 1973). As part of subsequent study by UW-Madison, Helmke et al. (1977) measured the concentrations of 22 metals in harbor and lake sediments and found then to be 3 to 4 fold higher in the clay sized (<2 u) fractions. This analysis of undisturbed red clay soil particles of the region demonstrated that the natural ranges of zinc and copper exceeded the 1973 EPA guidelines in the absence of pollution. Native soils contain between 140-230 mg/kg zinc and 65-88 mg/kg copper (Helmke et al. 1977).

None of the samples collected during the autumn of 1973 by UW-Madison (a total of 6) or subsequently by the US EPA during 1975, 1976 contained the concentration of mercury identified through the 1970 EPA or 1972-1973 National Biocentrics and UW-Superior analysis (National Biocentrics Inc., 1973). Although mercury concentration in bulk lake sediments did not exceed 0.24 mg/kg mercury, clay sized fractions contained as much as 4.22 mg/kg mercury. Comparisons were made between concentrations of 22 metals in standard red clay soils and clay sized sediments from harbor and lake sediments. For both harbor and lake sediments, ratios calculated by dividing the concentration of copper, zinc, chromium, mercury and arsenic in sediments by the concentrations in native soils exceeded one whereas, ratios calculated for metals not utilized by man were equal to one. The larger ratios show that harbor and lake sediments have been altered by man.

More recent measurements of contaminants in sediments have been directed at defining concentrations of toxic organic compounds particularly, polychlorinated biphenyls (PCBs). A limited survey conducted by EPA during

1976 showed pesticide concentrations were generally low but concentrations of PCBs were high in two of eight samples (exceeded 30 mg/kg; EPA, 1976). A more extensive survey conducted by the US Corps of Engineers during 1977 (Whiting, 1977) included 30 sampling sites and showed PCB concentrations did not exceed 0.5 mg/kg in harbor sediments and generally did not reach 0.1 mg/kg (dry weight).

AQUATIC LIFE

The potential for promoting human health problems or damage to the aquatic life community in the estuary and western end of Lake Superior, represent primary concerns in dredging and dredge spoil disposal. Some measurements on the aquatic life community have been performed to define the potential for the above problems and to determine the pollution status of harbor sediments. Identification of macroinvertebrates and measurement of species diversity suggests the bottom community is dominated by pollution tolerant forms in most zones. Surveys conducted by UW-Superior (National Biocentrics Inc., 1973) and the US EPA (EPA 1975) indicate that the presence of intolerant forms and high species diversity are limited to zones directly influenced by lake seiches. Abundance and distributions of macroinvertebrates have been shown to be positively correlated with the percentage of clay and silt sized particles in harbor sediments, which chemical analysis demonstrates contain the highest concentration of toxic metals (National Biocentrics Inc., 1973; Helmke _et al_., 1977). The question of whether zinc, or mercury in sediments are available to aquatic life in the harbor and western end of the lake has been studied by Helmke _et al_. (1976). Employing both chemical techniques which measure exchangeable and soluble zinc in sediments and comparisons between concentrations of zinc in sediments and in aquatic life sampled at various sites, Helmke _et al_. (1976) found zinc in sediments is not available to macroinvertebrates and does not appear to bioaccumulate in the aquatic food chain. Their limited analysis indicated that mercury in sediments may be available to macroinvertebrates and does bioaccumulate in the aquatic food chain. Analysis of mercury levels in sediments, macroinvertebrates (*Pontoporeia affinis*), sculpins and some other fish (white fish, lake trout and burbot) suggested the highest concentrations occur in animals at the top of the food chain. Upon exposure to available soluble forms of mercury and zinc, Magnuson *et al*. (1976) found behavioral changes occurred in *Pontoporeia affinis* resulting in increased susceptability of the animals to predators (sculpins).

SECTION 6

PROJECT FIELD SAMPLING

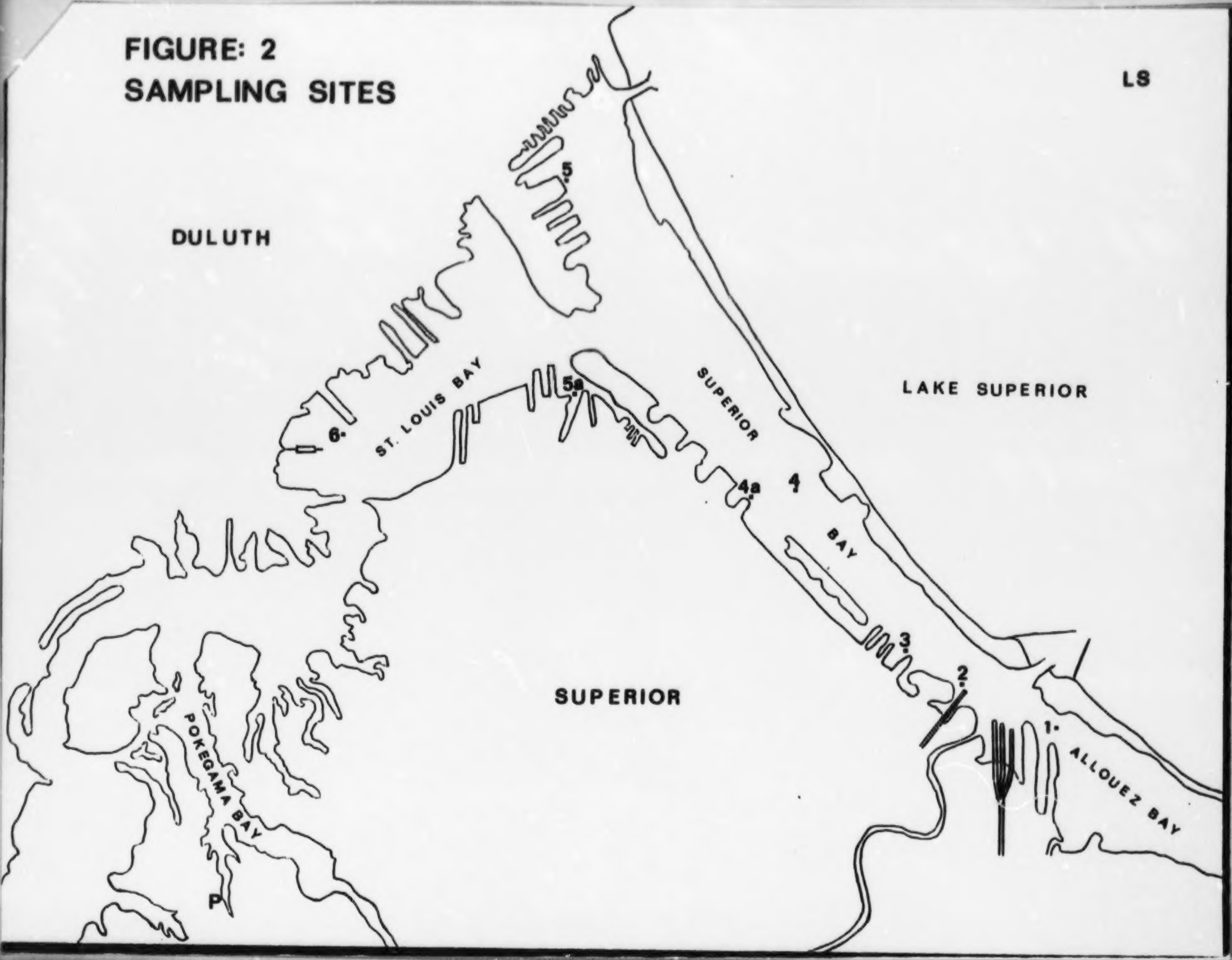
SAMPLING SITES

Samples were collected from six site areas in the Superior-Duluth Harbor during this research project. The sites were selected to provide a diversity of bottom sediment types and to allow comparison to previous sediment data. Reference samples were collected from the Pokegama Bay during 1977 and from Lake Superior in 1978. These reference samples were used as the controls in all bioassays (Section 8). Sampling locations are shown in Figure 2 and include:

1. Allouez Bay - Highly organic and clay type sediments are found in this region. Samples were collected from the northern edge of the eastern end of the dredged channel at a water depth of approximately 25 feet.
2. Superior Ore Dock Area - A clay-sand bottom dominates this area. Collection of samples at this site occurred approximately 50 yards northeast of the Northern Pacific ore dock at a depth of about 25 feet.
3. Lakehead Transshipment Terminal Area - The sediments were fairly high in volatile solids indicating substantial organic material. Sampling was done about 30 yards north of the end of the Lakehead Slip in approximately 24 feet of water.
4. Superior Municipal Sewage Treatment Plant - Organic sediments of domestic sewage origin were abundant. Samples were collected east of the treatment plant at the eastern edge of the ship channel in about 28 feet of water.

4a. Superior Municipal Sewage Treatment Plant - Sampling was done east of the treatment plant at the western edge of the ship channel

FIGURE: 2
SAMPLING SITES


LS
LAKE SUPERIOR
DULUTH
ST. LOUIS BAY
SUPERIOR BAY
SUPERIOR
ALLOUEZ BAY
POKEGAMA BAY
P
1.
2.
3.
4
4a
5.
5a
6.

at a depth of approximately 20 feet.

5. Cargill Inc. Elevator B - Sediments were a mixture of sand and organic material. Sampling of this site was located approximately 150 yards north of the end of the Cargill Elevator B slip in about 24 feet of water.

5a. Farmers Union Elevator - Sediment in this area contained organic matter of industrial origin. Samples were collected west of the end of the Farmers Union slip at a water depth of approximately 5 feet.

6. Minnesota Power and Light Company Generating Station - Fine sediments with substantial amounts of fly ash material dominated this region. This sampling location was approximately 700 yards east - northeast of the power plant in about 19 feet of water.

P. Pokegama Bay - Organic sediment minimally affected by human development occurred in this area. Sampling of this reference site was done at the southern end of the west branch of Pokegama Bay. Samples were collected in about 4 feet of water.

L.S. Lake Superior - The sediment at this location contained substantial amounts of eroded silt and clay. Collection of samples occurred approximately 6 miles north of the Superior Entry to the Duluth-Superior Harbor at a water depth of about 95 feet. The Lake Superior site replaced Pokegama Bay as the reference site in 1978 because chemical analysis of Pokegama Bay sediments showed high concentrations of chemical oxygen demand and mercury.

SEDIMENT, WATER AND CHIRONOMIDS SAMPLING

Samples of sediment and water from the Duluth-Superior harbor area and the Lake Superior site were collected using the University of Wisconsin-Superior RV GULL. Each sampling site was located according to shoreline structure references and water depth. During 1977, seven cores were obtained at each location employing a Benthos type 217 gravity corer containing cellulose acetate butyrate liners 4 foot (1.2 m) in length and 2.5 inches (6.3 cm) inside diameter. Upon obtaining a core, the ends of the core liner were stoppered and it was stored upright in an insulated box type holder cooled with ice. The core liners contained about 8 to 18 inches (20.3 to 45.7 cm) of harbor sediment with the remainder of the liner filled with overlying harbor water. The cores were utilized in chemical analyses and bioassay tests (usually the day following sampling).

During 1977, portions of the overlying water from several of the cores were siphoned into glass or polyethylene containers for later use in chemical analysis and enzyme activity tests. Preservatives were added to sub-samples of the overlying water and subsequently analyzed for ammonia, total sulfide and total phenols. The sub-samples were cooled in an ice chest.

Sediment samples were also obtained at each site employing a Ponar dredge. Portions of these sediment samples were transferred to glass bioassay chambers for tests using Daphnia magna placed in water above the sediments and using the burrowing fauna Hexagenia limbata. These bioassay tests commenced the day following this sampling.

Portions of the sediment were rinsed through a 35 mesh size stainless steel sieve and the Chironomids retained by the sieve were placed in a glass vial containing harbor water. A second method consisted of hand mixing sediment with harbor water in a metal wash basin and collecting the animals appearing on the water surface. The Chironomids were cooled in an ice chest and transported to the laboratory for weighing and freezing prior to chemical analysis.

During 1978, a similar sampling program was carried out for harbor sites except that water overlying the cores was not analyzed and a fewer number of cores were obtained at each site. Dredge sediment was used from only two of the harbor sites and the Lake Superior site for Hexagenia limbata toxicity tests.

The reference site used in bioassay tests during 1977 was the Pokegama Bay slough. Core samples were obtained by inserting the cellulose butyrate acetate core liner into the sediments at a water depth of 4 feet (1.2 m). During 1978, the Lake Superior site was used as the reference site in bioassay tests.

The sediment and water samples were collected on the dates listed in Table 1.

HEXAGENIA LIMBATA

Two locations were used during the study for collecting *Hexagenia limbata*. Animals used in toxicity tests conducted during 1977 were collected from a bay on Rainy Lake in northern Minnesota (International Falls, Minnesota). The second source of *Hexagenia limbata* was Ox Creek in northern Wisconsin. The Ox Creek *Hexagenia* were used in 1978 bioassay and bioaccumulation studies.

The collection procedure consisted of obtaining the upper layer of sediment with a shovel, transferring the sediment to screen boxes and rinsing the sediment with water from the collecting location. The animals were removed from the screen box as they were exposed using plastic spoons and placed in water in a cooler. During transport to the laboratory, the water was aerated and kept at about 15 C. Sediment was collected from the sampling location for use in storing the animals prior to their use in the tests.

PONTOPOREIA AFFINIS

The *Pontoporeia* were collected from Lake Superior at a 75 foot (29.9 m) water depth at a location north of the Superior entry to the Superior Harbor. The location was approximately two miles from the entry.

The upper layer of sediment was obtained with a Ponar dredge and placed in wash basins. Water was added and mixed with the sediment to

TABLE 1. Sediment and Water Sample Collection Dates

1977 Sampling

Site	Date Collected	Reference Site	Date Collected
1	June 12	Pokegama	June 12
2	June 5	Pokegama	June 5
3	July 10	Pokegama	July 10
3R	October 14	Lake Superior	October 12
4	July 17	Pokegama	July 17
4R	October 21	Lake Superior	October 12
5a	July 24	Pokegama	July 24
6	June 26	Pokegama	June 26
6R	August 14	Pokegama	August 14
L.S.	August 7	Pokegama	August 7
Pokegama	June 19	----	----
1978 Sampling			
1	June 2	Lake Superior	June 2
2	June 23	Lake Superior	June 17
2	July 14	Lake Superior	July 6
3	June 17	Lake Superior	June 17
4	June 8	Lake Superior	June 2
4a	July 14	Lake Superior	July 6
5	June 23	Lake Superior	June 17
5	July 7	Lake Superior	July 6
5	July 21	Lake Superior	July 20
6	July 7	Lake Superior	July 6
6	July 21	Lake Superior	July 20

form a slurry. The slurry was poured through a screen box into a second basin containing lake water. The animals were floated away from the screen, collected with a plastic spoon and placed in a four liter plastic bottle containing lake water. The container was placed in a cooler and transported to the laboratory for storage.

LEPOMIS MACROCHIRUS

The bluegills (Lepomis macrochirus) used in the fish cough response bioassays were collected from Bass Lake, a small lake located in Bayfield County near Delta, Wisconsin.

The fish were obtained by seining the inshore areas of the lake. Only those bluegills that were between 7 and 11 cm in length were retained. The captured fish were placed in 30 gallon metal containers (lined with polyethylene) that had previously been filled with lake water. During transportation to the laboratory the water was aerated and kept in the dark to minimize temperature changes.

SECTION 7

CHEMICAL CHARACTERIZATION OF SAMPLES AND WATER-SEDIMENT SYSTEM PREPARATION

SEDIMENT-WATER SYSTEMS UTILIZED

The sediment-water systems chosen for chemical characterization primarily consisted of the same systems subsequently used in behavioral response and acute toxicity bioassay tests. The systems analyzed were sediment, water overlying the sediment, sediment interstitial water, elutriate water and "generated pore water". The specific chemical parameters investigated were chosen to provide a comprehensive survey (within budget and time limitations) of those chemical species indicative of the overall quality of the systems.

TREATMENT OF CORE SAMPLES

Background

The procedures utilized in obtaining the various sediment-water systems from the core samples were designed to maintain sample integrity and to provide sufficient amounts for chemical and biological tests.

A number of methods for extracting interstitial water from sediments have been employed by investigators (Presley et al., 1967; Duchart et al., 1973; Robbins and Gustinus, 1976). The exposure of sediments to air, temperature fluctuations and mechanical perturbations can alter the chemical nature of the interstitial water (Bray et al., 1973; Troop et al., 1974; Bischoff et al., 1970). For collecting large volumes of interstitial water, the method used in this investigation involved high speed centrifugation of the sediment at 4°C followed by collection of the liberated water under a nitrogen atmosphere. After the initial separation of the water from the sediment, the water was recentrifuged, decanted and utilized in chemical and biological tests. The water was not filtered and consequently it contained fine suspended solids. The interstitial water, containing this fine suspended material, is considered representative of material released to the overlying water in the event of dredging operations.

Elutriate water was prepared from sediment mixed with Lake Superior water according to published procedures (EPA/Corps of Engineers, 1977). However the elutriate water was not filtered to avoid possible loss of toxic gases to the air and possible loss of metals or organics on the filter material. The liquid phase was obtained by using only high speed centrifugation as the means of separating suspended solids from the aqueous phase. Two elutriate water systems were prepared for each sediment site (summer of 1977). One system used sediment exposed to air while the second system employed sediment which had been kept under nitrogen prior to elutriate water preparation.

An attempt was made to arrive at an estimate of the amounts of chemical species which could potentially be flushed out of the sediments under vigorous mixing conditions. Sediment (unexposed to air) was repeatly extracted with Lake Superior water over a period of time to obtain "generated pore water". The chemical characteristics of the generated pore water gives a measure of the potential availability of chemical species to the water phase.

Procedure

During the summer of 1977, the sediment core samples were extruded and used to prepare the various sediment-water systems usually on the day following sampling. The cores had been stored upright in the plastic liners under lowered temperature conditions (<12 C) in the dark.

The cores were treated by the scheme shown in Figure 3. Nearly all of the overlying water was siphoned from the top of the sediments and discarded (overlying water had been collected at the time of sampling). Measurements of pH and Eh were made 2 cm below the surface. The stopper was removed from the bottom of the core and replaced with one which tightly fit inside the liner. The upper portion of the liner was placed in a glove bag and the stopper and core were forced toward the top of the liner. The residual overlying water and upper 1 cm of the core was discarded.

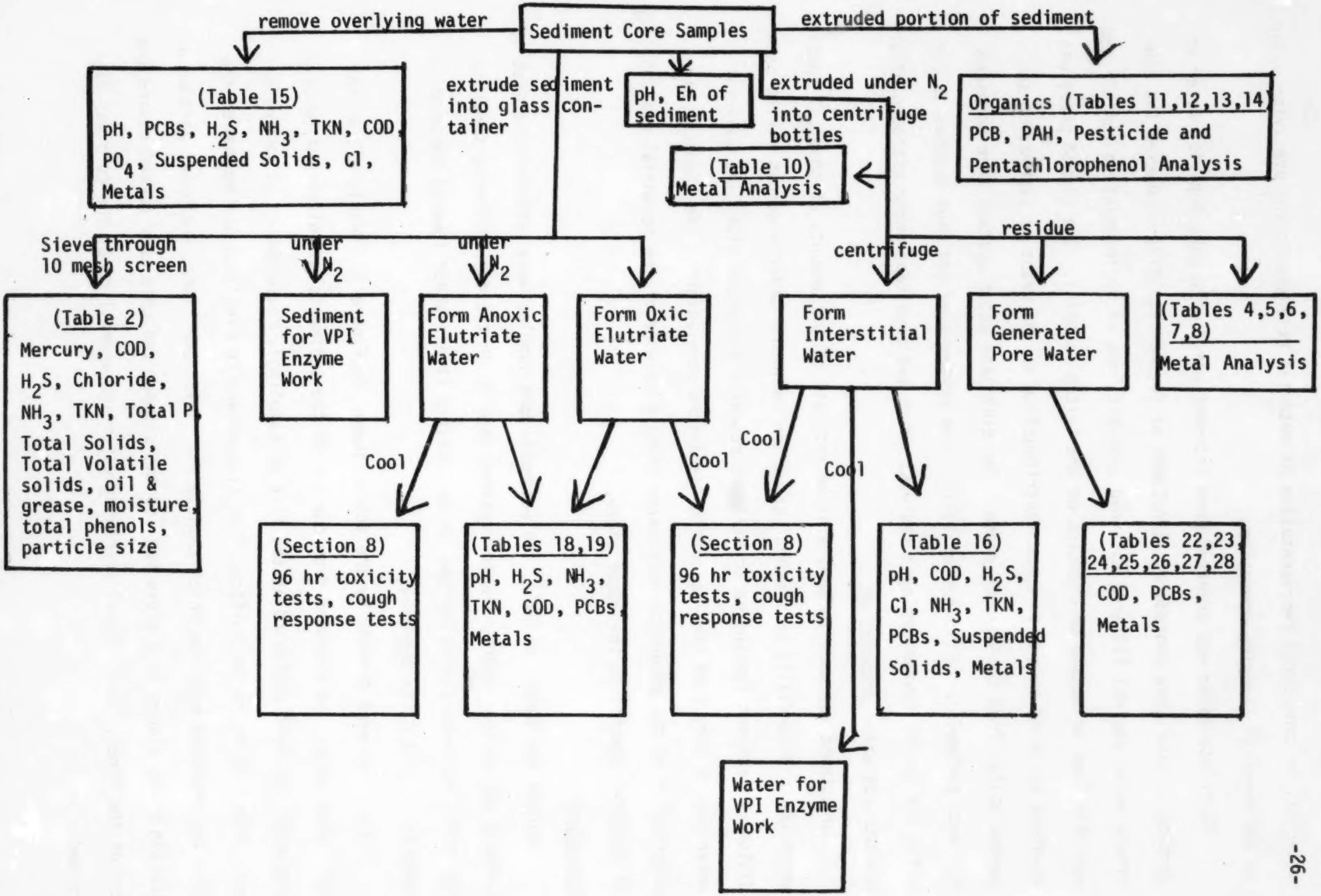

Sediment Core Samples
remove overlying water
extruded portion of sediment
(Table 15)
pH, PCBs, H_2S, NH_3, TKN, COD, PO_4, Suspended Solids, Cl, Metals
extrude sediment into glass container
pH, Eh of sediment
extruded under N_2 into centrifuge bottles
Organics (Tables 11,12,13,14)
PCB, PAH, Pesticide and Pentachlorophenol Analysis
(Table 10)
Metal Analysis
Sieve through 10 mesh screen
under N_2
under N_2
centrifuge
residue
(Table 2)
Mercury, COD, H_2S, Chloride, NH_3, TKN, Total P, Total Solids, Total Volatile solids, oil & grease, moisture, total phenols, particle size
Sediment for VPI Enzyme Work
Form Anoxic Elutriate Water
Form Oxic Elutriate Water
Form Interstitial Water
Form Generated Pore Water
(Tables 4,5,6, 7,8)
Metal Analysis
Cool
Cool
Cool
Cool
(Section 8)
96 hr toxicity tests, cough response tests
(Tables 18,19)
pH, H_2S, NH_3, TKN, COD, PCBs, Metals
(Section 8)
96 hr toxicity tests, cough response tests
(Table 16)
pH, COD, H_2S, Cl, NH_3, TKN, PCBs, Suspended Solids, Metals
(Tables 22,23, 24,25,26,27,28)
COD, PCBs, Metals
Water for VPI Enzyme Work

Figure 3. General Treatment of 1977 Superior-Duluth Harbor Core Samples

Sediment from the core was transferred to 250 ml stainless steel centrifuge bottles using a glazed ceramic spoon. Each cup was filled within about three-fourths of its capacity (about 180 g of sediment). One core was used to fill two or three centrifuge bottles. The bottles were capped, removed from the glove bag and centrifuged for 12 minutes at 10,000 rpm and 4°C (IEC model B-20A refrigerated centrifuge). The centrifuging provided a force of 10,000 x g at the lower end of the bottle decreasing to 3,500 x g at the upper end.

The cups were returned to the glove bag where the caps were removed and the interstitial water decanted into teflon bottles. Since a substantial amount of particulate matter was present in the water, it was placed in 250 ml polycarbonate centrifuge bottles and capped. The particulate-water suspension was recentrifuged at 14,000 rpm (25,000 x g at the lower end of the bottle progressing to 8000 x g at the upper end) for 15 minutes. The resulting interstitial water was decanted into teflon bottles (exposed to air) and stored in a refrigerator. After removal of interstitial water, portions of the sediment from several cores were transferred under nitrogen to a plastic bottle for subsequent metal analysis.

Other portions of the sediment were removed from cores and placed in two glass bottles. One bottle was used for general chemical sediment analyses while the second was sent under an N_2 atmosphere to Virginia Polytechnic Institute and State University, Blacksburg, Va (along with a subsample of the intersitial water) for use in enzyme activity tests. Another sample (about 1000 g) was placed on aluminum foil, air dried, and used in organic chemical analysis. Other portions of the sediment were saved for preparation of elutriate water.

SEDIMENT ANALYSIS

The sediment samples obtained during the summer of 1977 were subjected to extensive inorganic and organic analysis. This analysis included a number of general physical and chemical tests, specific metal determinations, PCB, pentachlorophenol and polycyclic aromatic hydrocarbon measurements. A much more limited sediment analysis was carried out on sediments sampled during the summer of 1978.

General Physical and Chemical Sediment Tests

Sediment samples obtained from several cores for a site were prepared for general analysis using EPA procedures (Fuller, 1969). The sediment was passed through a 10 mesh (approximately 2 mm pore size) sieve and collected in a large beaker. It was either blended or stirred (in the case of sediments with high water content) and divided into subsamples. The subsamples were analyzed for chemical oxygen demand (COD), ammonia, Kjeldahl nitrogen, moisture content, volatile solids, total phosphorus, total phenols, oil and grease and total sulfide using EPA methods (Fuller, 1969). These procedures are summarized in Appendix A.

Particle size analyses were performed by the pipet-sedimentation method described by Royse (1970). Total and organic mercury were determined by the procedure of Olson *et al*. (1975). This method involved sediment digestion in a concentrated mixture of HNO_3 and H_2SO_4, oxidation of organic matter with $KMnO_4$, reduction of mercury with stannous sulfate followed by atomic absorption measurements.

The sediment samples obtained during the summer of 1978 were analyzed only for pH, Eh and particle size. The results of these general sediment analyses are given in Tables 2 and 3.

Preparation of Sediment Extracts for Metal Analysis

The characterization of the sediments for concentrations of numerous metals was carried out using a procedure which generally followed the selective extractive methods of Engler *et al*. (1974) and Chen *et al*. (1976). The selective extraction of metals results in their classification as to availability to various chemical extractants.

TABLE 2

GENERAL SEDIMENT ANALYSIS-1977

	SITE								
Parameter*	1	2	3	4	5a	6	6R**	LS	Pokegama
pH†	6.3	6.1	6.2	6.6	6.3	6.5	6.9	7.1	6.1
Eh† (mv)	90	85	60	80	45	55	75	110	80
COD	99,000 104,000	35,000 31,000	85,000 91,000	136,000 138,000	57,000 62,000	133,000	168,000	34,000	168,000
Ammonia	46 79	5.3	500 720	1900 1400	500 620	140 139	580 690	1840 1230	107 167
TKN	660 675	270 205	3000 4300	7200 7400	1700 2400	1200 1200	4400 4000	5600 4900	129 220
Total S	26 43	2.4 3.1	1.8 1.6	240 220	89 82	87 67	300 210	11.2 6.2	23 21
Total P	130	75 75	83	1600 820	1400 1050	110	1700 1400	1500 2000	78
Oil and Grease	630 150	1700 1700	2200 4300	9600 12300	3700 3500	7200 2900	4500 3500	5600 4800	1120 880
Total Phenols	2.0	0.2	2.2 2.9	1.7 1.5	1.8 1.6	2.0	1.2 0.8	2.5 3.2	1.5
Organic N	610 600	265 200	2500 3600	5300 6000	1200 1750	1060 1110	3800 3300	3700 3600	22 53
Total Mercury	0.66	0.08	0.64	0.77	0.13	0.23	0.23	0.04	0.92

---

* Values are in mg/kg of dry sediment except for pH, Eh.
**R denotes a repeat sampling of site 6.
† Obtained about 2 cm below surface.

TABLE 2. (continued)

GENERAL SEDIMENT ANALYSIS-1977

Parameter	SITE 1	2	3	4	5a	6	6R	LS	Pokegama
Total Solids (%)	35.7	61.6	45.2	36.6	63.6	51.6	43.5	45.0	45.0
Total Volatile Solids (%)	11.2	5.1	9.4	11.9	6.4	9.7	11.1	4.7	12.5
% Sand (>62.5μ)	4.2 5.5	37.2	16.0 15.6	5.1 4.3	58.6 57.4	30.5 36.5	20.5 17.0	9.0 10.5	4.7 4.1
% Silt (3.9-62.5μ)	36.4 35.8	42.9	39.1 37.8	45.0 40.7	23.4 21.0	45.7 31.6	46.4 55.9	55.2 53.4	36.8 38.8
% Coarse Clay (0.98-3.9μ)	19.0 18.5	6.8	17.8 16.2	21.4 18.3	7.9 7.2	13.3 21.5	17.2 12.7	12.2 13.5	39.8 21.7
% Fine Clay (<0.98μ)	40.3 40.2	9.7	27.1 30.4	28.5 36.6	10.2 13.0	10.5 10.3	15.9 14.4	23.7 23.6	18.5 35.4

TABLE 3

GENERAL SEDIMENT ANALYSIS-1978

	SITE								
Parameter	1	2	2R	3	4	4a	5	6	L.S.
pH*	6.5	--	6.3	6.4	6.5	6.5	--	6.3	6.5
Eh* (mv)	101	--	104	76	36	38	--	-49	113
Total Solids (%)	52.4	52.1	--	40.0	38.7	--	61.5	50.4	--
Total PCBs**	0.31	0.61	--	1.6	1.0	--	0.85	0.77	0.33
% Sand	13.5 ±2.0	2.6 ±0.5	--	3.5 ±2.9	2.6 ±2.5	--	62.2 ±4.1	4.4 ±3.8	1.12 ±0.2
% Silt	40.7 ±3.6	40.7 ±6.5	--	32.6 ±11.4	23.7 ±10.6	--	22.1 ±1.0	53.7	72.7 ±2.7
% Course Clay	14.8 ±1.2	17.2 ±2.4	--	21.5 ±3.5	24.8 ±0.2	--	5.6 ±1.0	11.7	7.4 ±0.2
% Fine Clay	31.0 ±2.9	39.5 ±4.7	--	42.4 ±10.7	49.9 ±13.0	--	10.0 ±2.2	30.7 ±4.3	21.1 ±0.9

* Obtained about 2 cm below surface.

**Expressed as mg/kg of dry sediment.

Sediment subsamples were obtained under a nitrogen atmosphere from the upper layers of sediment in the centrifuge bottles from which the interstitial water had been removed. Total metal content analyses of sediment from two of the sites were carried out on uncentrifuged sediment still containing interstitial water. The classification of metals according to their extractability and procedures employed are summarized below.

Exchangeable Phase Metals --

The exchangeable metals are obtained by extraction with ammonium acetate. This treatment removes metals which are largely adsorbed to mineral and organic surfaces. The metals in this phase are believed to be in rapid equilibrium with metals in the interstitial water phase. They are available to replenish metals which are removed biologically and chemically from the interstitial water.

150 ml of 1.0 M deaerated ammonium acetate solution (deaerated by bubbling N_2 through the solution) was added (under a N_2 atmosphere), to about 22 to 30 g dry weight of the centrifuged sediment from which the interstitial water had been extracted. The sediment was put into sealable centrifuge cups. Since the sediment was not completely dry, part of the sediment was used to determine the percent dry weight of the sample. The sealed plastic centrifuge bottle was shaken for 90 minutes with a mechanical shaker. After shaking, the exchangeable phase was separated by centrifugation followed by filtration through 0.45 micron filters. Duplicate samples were usually run through the entire metal extraction procedure.

Easily Reducible Phase Metals --

The metals in the easily reducible phase are obtained by using hydroxylamine hydrochloride and a dilute solution of nitric acid as the extractants. This treatment removes metals which are mainly associated with hydrous oxides of manganese. Four to five gram (dry weight) subsamples were taken from the residue to the previous extraction (exchangeable phase) and washed with deionized water. A portion was used to determine the dry weight of the sample. The washed residue was extracted with 200 ml of 0.1 M hydroxylamine hydrochloride in 0.01 M HNO_3 solution by shaking the mixture for 45 minutes with a mechanical shaker. The extract was separated by centrifuging and filtering the supernatant. The aqueous phase was acidified to 0.2% HNO_3 for metals analysis.

Organic and Sulfide Phase Metals --

Treatment of residue from the easily reducible phase with concentrated hydrogen peroxide and acidic ammonium acetate releases metals which are associated with metal sulfides and the organic fraction of the sediment. Also manganese from MnO_2 not extracted as part of the easily reducible phase is obtained. The organically complexed metals vary in stability and bioavailability but may be subject to release from the sediments by microbial degradation.

The residue from the easily reducible phase was washed with deionized water. A subsample of 1 to 2 g (dry weight) was weighed into a plastic centrifuge cup and treated with 3 ml of 0.02 M HNO_3 and 5 ml of acidified 30% H_2O_2 (acidifed to about pH 2). A portion of the initial washed residue was used in determining the dry weight of the sample. The mixture was heated in a water bath at $85^{\circ}C$ for 5 hours. After 2 hours of heating, another 2 to 3 mls of acidified 30% H_2O_2 was added. After the mixture was allowed to cool to room temperature, 25 ml of 1.0 M ammonium acetate in 6% HNO_3 was added and the sample shaken in a mechanical shaker for 30 minutes. Residual solids were separated by centrifugation and filteration.

Moderately Reducible Phase Metals --

Extraction of the residue from the previous step with hydroxylamine hydrochloride in acetic acid liberates reduced iron and metals associated with hydrous iron oxides. These metals could be released from the sediments to the overlying water under reducing conditions.

The entire residue from the previous step was washed with deionized water and treated with 40 ml of 0.04 M hydroxylamine hydrochloride in 25% acetic acid. The resulting mixture was shaken well, heated at 100°C for 3 hours in a water bath. After 3 hours, the mixture was cooled to room temperature and the extract separated as in previous steps.

Residual Phase Metals --

The moderately reducible phase residue contains weathered minerals which is generally the location of the largest amounts of metals in the sediment. The metals are in the inner layer positions of clay minerals or within the mineral crystal lattice. These metals are stable and in a biologically unavailable form.

The residue from the previous extraction was washed with distilled water (which was discarded) and a 0.5 to 0.8 g (dry weight) portion was weighed into a 50 ml teflon beaker. (A portion was used to determine the dry weight in the sample.) The sample was digested with a mixture of 10 ml concentrated HF, 5 ml concentrated HNO_3, and 1 ml concentrated $HClO_4$ in a heated sand bath at about 170°C (surface temperature of sand), and evaporated to near dryness. The residue was dissolved in 5 ml concentrated HCl (heating was usually necessary). If some undissolved residue remained, about 20 ml deionized water was added and the solution was filtered through a 0.45 micron filter. The filtrate was stored in a plastic bottle. (If after the original digestion, and upon addition of the HCl, the residue completely dissolved, then deionized water was added and the sample was brought to a volume of 50 ml. At this point the sample was ready to be analyzed for metals.)

The undissolved residue was scraped and washed off the filter into a platinum evaporating dish. The platinum evaporating dish, containing the residue and wash water, was dried overnight at 100°C, cooled, and weighed. Enough sodium carbonate was added to completely cover the dry residue (1-5 ratio) and the dish and its contents were placed in a muffle furnace at about 900°C. As the Na_2CO_3 melted, the dish was swirled to insure complete mixing of residue and Na_2CO_3. The platinum dish was removed from the furnace and allowed to cool to room temperature. When cool, 1 ml of deionized water was added and the mixture heated slowly on a hot plate at low setting. Concentrated HCl was carefully added until the sample was acidic. The mixture was stirred and heated on the hot plate until all the solid dissolved. At this time, the filtrate from the original digestion was added and the combined solution evaporated to near dryness. Finally, deionized water was added to bring the solution to a 50 ml volume.

Metal Analysis Procedures

Atomic absorption spectroscopy was used in the metal analysis. Flame absorption methods (employing direct aspiration of the aqueous phase extracts) were used for those samples containing relatively high metal concentrations (generally 0.1 to 1.0 ppm or above in the extracts). For those extracts with metal concentrations below those detectable by direct aspiration into the flame, a graphite furnace was used. The instruments utilized were the Perkin Elmer Model 306 atomic absorption system and the HGA 2100 graphite furnace with background correction. Pyrolyzed graphite tubes were used to increase detection limits (Manning and Ediger, 1976).

Metal Analysis Results

The concentrations of metals found in the various phases of the sediment are given in Tables 4 through 8. These values are computed on a dry weight basis. The concentrations are corrected for any weight losses occurring during the extraction steps and for reagent blanks.

TABLE 4

Metal Concentrations in Exchangeable Phase of Sediment Samples (mg/kg)-1977

	As	Cd	Cr	Co	Cu	Fe	Pb	Mn	Ni	Se	Zn
Site 1	<0.08	<0.40	<4.1	<1.6	<0.40	170	<1.6	170	<1.6	<0.16	<0.40
Site 1	<0.08	<0.39	<3.9	<1.5	<0.39	170	<1.5	110	<1.5	<0.15	<0.39
Site 2	<0.04	<0.17	<1.7	<0.7	<0.17	3.6	<0.7	105	<0.7	<0.07	<0.17
Site 2	<0.06	<0.27	<2.7	<1.1	<0.27	---	<1.1	171	<1.1	<0.11	<0.27
Site 3	<0.05	<0.23	<2.3	<0.9	<0.23	32.2	<0.9	61.9	<0.9	<0.09	<0.23
Site 3	<0.05	<0.26	<2.6	<1.0	<0.26	29.2	<1.0	48.2	<1.0	<0.10	<0.26
Site 4	<0.05	<0.26	<2.6	<1.0	<0.26	<1.0	<1.0	90.7	<1.0	0.10	<0.26
Site 4	<0.06	<0.31	<3.1	<1.2	<0.31	1.2	<1.2	133	<1.2	<0.12	<0.31
Site 5a	<0.05	<0.22	<2.2	<0.9	<0.22	20.7	<0.9	27.0	<0.9	<0.09	<0.22
Site 5a	<0.05	<0.23	<2.3	<0.9	<0.23	11.7	<0.9	31.5	<0.9	<0.09	<0.23
Site 6	<0.08	<0.41	<4.1	<1.6	<0.41	7.8	<1.6	96.7	<1.6	<0.16	<0.41
Site 6	<0.08	<0.40	<4.0	<1.6	<0.40	9.1	<1.6	93.7	<1.6	<0.16	<0.40
Site 6R	<0.04	<0.19	<1.9	<0.7	<0.19	4.0	<0.7	100	<0.7	<0.07	<0.19
Site 6R	<0.04	<0.21	<2.1	<0.8	<0.21	4.5	<0.8	124	<0.8	<0.08	<0.21
Site LS	<0.07	<0.33	<3.3	<1.3	<0.33	17.8	<1.3	65.8	<1.3	<0.13	<0.33
Site LS	<0.06	<0.29	<2.9	<1.2	<0.29	11.1	<1.2	59.5	<1.2	<0.12	<0.29

TABLE 5

Metal Concentrations in Easily Reducible Phase of Sediment Samples (mg/kg)-1977

	As	Cd	Cr	Co	Cu	Fe	Pb	Mn	Ni	Se	Zn
Site 1	<0.45	<2.2	<22	<8.9	<2.2	840	<8.9	97	<8.9	<0.89	<2.2
Site 1	<0.45	<2.2	<22	<8.9	<2.2	660	<8.9	76	<8.9	<0.89	<2.2
Site 2	<0.36	<1.8	<18	<7.2	<1.8	420	<7.2	94	<7.2	<0.72	<1.8
Site 2	<0.26	<1.3	<13	<5.2	<1.3	270	<5.2	99	<5.2	<0.52	<1.3
Site 3	<0.41	<2.1	<21	<8.2	<2.1	1400	<8.2	110	<8.2	<0.82	3.3
Site 3	<0.48	<2.4	<24	<9.6	<2.4	1400	<9.6	77	<9.6	<0.96	3.9
Site 4	<0.46	<2.3	<23	<9.1	<2.3	2800	<9.1	110	<9.1	<0.91	7.3
Site 4	<0.46	<2.3	<23	<9.1	<2.3	3100	<9.1	130	<9.1	<0.91	5.9
Site 5a	<0.45	<2.3	<23	<9.0	<2.3	730	<9.0	54	<9.0	<0.90	2.3
Site 5a	<0.43	<2.2	<22	<8.6	<2.2	900	<8.6	56	<8.5	<0.86	<2.2
Site 6	<0.50	<2.5	<25	<10.0	<2.5	710	<10.0	120	<9.9	<1.00	13.0
Site 6	<0.44	<2.2	<22	<8.8	<2.2	650	<8.8	130	<8.8	<0.88	11.9
Site 6R	<0.53	<2.6	<26	<10.5	<1.3	3800	<10.5	170	<10.5	<1.05	12.6
Site 6R	<0.56	<2.8	<28	<11.2	<1.3	4200	<11.2	180	<11.2	<1.12	12.9
Site LS	<0.54	<2.7	<27	<10.8	<2.6	1500	<10.8	66	<10.8	<1.08	<2.7
Site LS	<0.50	<2.5	<25	<9.9	<2.8	1900	<9.9	76	<9.9	<0.99	<2.5

TABLE 6

Metal Concentrations in Organic Phase of Sediment Samples (mg/kg)-1977

	As	Cd	Cr	Co	Cu	Fe	Pb	Mn	Ni	Se	Zn
Site 1	---	<0.79	<7.9	3.1	18.1	880	22.0	165	5.5	---	26.7
Site 1	2.4	<0.67	<6.7	3.1	17.1	860	21.5	139	<2.7	<0.27	23.8
Site 2	1.8	<0.98	<9.8	3.9	14.4	690	3.9	93.6	<3.9	<0.39	25.2
Site 2	2.2	<1.0	<10.0	<4.0	19.5	990	8.0	84.4	<4.0	0.80	18.1
Site 3	4.4	1.1	<7.9	4.7	39.9	4600	53.8	85.9	4.9	0.47	87.8
Site 3	4.2	6.3	<8.8	7.0	49.9	5300	68.3	103	69.3	0.53	155
Site 4	3.8	1.4	<8.0	4.8	47.8	6700	83.4	155	17.2	0.32	152
Site 4	5.6	1.1	<8.0	6.4	42.8	9500	84.9	155	8.1	0.64	133
Site 5a	2.5	<0.95	<9.5	<3.8	15.2	1900	41.8	69.7	8.2	<0.38	31.3
Site 5a	2.3	0.78	<7.8	5.1	29.6	3400	63.9	89.1	2.5	<0.31	75.0
Site 6	3.2	1.1	<7.1	6.6	19.5	900	41.0	84.7	404	0.28	88.1
Site 6	5.2	0.75	<7.5	7.1	23.3	1000	50.8	102	209	0.30	88.5
Site 6R	5.1	1.6	<9.1	4.9	30.1	6600	77.4	134	22.1	0.36	72.0
Site 6R	9.8	1.7	<8.7	4.7	33.5	6400	91.4	133	41.9	0.87	178
Site LS	1.8	<0.82	<8.2	5.4	21.2	3900	6.5	73.2	127	<0.33	53.8
Site LS	2.2	<0.69	<6.9	4.6	20.7	4000	5.5	73.9	15.9	0.28	19.6

TABLE 7

Metal Concentrations in Moderately Reducible Phase Sediment Samples (mg/kg)-1977

	As	Cd	Cr	Co	Cu	Fe	Pb	Mn	Ni	Se	Zn
Site 1	<0.20	<1.0	<10.1	5.4	10.8	2500	<4.1	62.9	8.7	<0.41	22.7
Site 1	0.34	<0.87	<8.7	4.0	8.8	1400	<3.5	60.8	8.7	<0.35	20.0
Site 2	<0.18	<0.91	<9.1	5.4	9.2	1700	<3.6	61.7	6.4	<0.36	11.4
Site 2	0.32	<0.82	<8.2	6.0	14.6	1600	<3.3	57.2	8.8	<0.33	14.9
Site 3	0.20	<1.02	<10.2	<4.1	9.2	2900	<4.1	48.8	4.9	<0.41	19.1
Site 3	0.68	<1.1	<11.2	8.3	11.9	4000	<4.5	63.0	10.3	<0.45	29.9
Site 4	0.38	<0.95	<9.5	<3.8	10.6	4700	<3.8	60.9	5.9	<0.38	32.7
Site 4	0.38	<0.95	<9.5	<3.8	10.6	4900	<3.8	62.8	5.1	<0.38	30.4
Site 5a	1.02	<1.3	<12.7	5.1	8.8	4700	<5.1	61.1	14.8	<0.51	34.6
Site 5a	0.63	<1.0	<10.4	<4.2	7.9	4600	<4.2	62.5	10.4	<0.42	40.6
Site 6	0.37	<0.93	<9.3	4.3	6.0	3000	<3.7	53.8	59.3	<0.37	31.7
Site 6	0.78	<0.98	<9.8	3.9	6.4	2300	<3.9	60.8	70.6	<0.39	33.6
Site 6R	<0.24	<1.2	<11.9	6.5	8.2	8400	<4.8	117	12.9	<0.48	51.7
Site 6R	0.48	<1.2	<11.9	4.8	12.7	7600	<4.8	90.9	7.4	<0.48	41.4
Site LS	0.63	<1.1	<10.6	4.9	20.2	5500	<4.2	90.0	33.8	<0.42	42.1
Site LS	0.36	<0.90	<9.0	5.9	18.8	4200	<3.6	70.0	8.3	<0.36	16.3

TABLE 8

Metal Concentration in Residual Phase of Sediment Samples (mg/kg)-1977

	As	Cd	Cr	Co	Cu	Fe	Pb	Mn	Ni	Se	Zn
Site 1*	2.6	<4.4	<43.9	17.6	17.1	8,800	<17.6	149	23.7	<1.8	35.1
Site 1*	4.9	<4.9	<48.8	22.5	26.7	9,400	<19.5	186	26.4	<2.0	47.9
Site 2*	5.3	<4.4	<43.9	20.2	14.9	18,100	<17.6	167	<17.6	<1.8	34.3
Site 2*	3.3	<4.1	<41.4	24.8	18.3	17,600	<16.6	166	<16.6	<1.7	34.8
Site 3	24.8	6.2	176	70.3	57.5	46,100	51.7	393	59.9	32.1	57.9
Site 3	25.4	8.5	182	61.8	70.5	41,300	48.5	376	52.1	29.1	55.7
Site 4	24.1	8.6	<43.1	44.0	30.1	38,900	<17.2	285	43.1	13.8	42.3
Site 4	18.7	4.3	<42.5	45.9	16.6	42,000	<17.0	272	145	11.1	40.8
Site 5a	22.4	<6.6	<65.9	<26.4	23.7	31,100	<26.4	316	<26.4	5.3	42.2
Site 5a	10.9	9.1	<45.6	24.6	42.9	39,300	<18.2	252	<18.2	21.9	58.4
Site 6*	7.3	<3.7	132	42.5	72.9	11,500	36.7	198	45.5	13.2	51.3
Site 6*	12.2	9.3	137	76.2	34.4	11,300	50.3	208	63.3	2.2	67.6
Site 6R	15.4	<4.0	<40.5	25.9	28.3	47,100	20.2	373	<16.2	2.4	80.2
Site 6R	13.1	<4.4	<43.6	43.6	57.6	47,200	<17.5	355	<17.5	8.7	59.4
Site LS	28.4	<4.9	<48.9	36.2	35.3	42,000	<19.6	438	34.3	19.6	69.5
Site LS	19.4	5.1	<51.1	<42.9	30.6	39,100	<20.4	434	<20.4	10.2	68.4

*Samples were not carried through the Na_2CO_3 fusion step.

The sediment samples analyzed by this selective extractive method were obtained from the upper layers of centrifuged samples. This material is enriched in smaller particles which would settle in water at the slowest rate after suspension due to a disturbance of the sediment. Consequently, it should contribute a more important environmental effect (compared to the total sediment) when subjected to dredging. Therefore the upper layer of the residue (obtained after removal of the interstitial water) was utilized in the selective extractive procedure.

The enrichment of metals in the upper layer of this residue is illustrated by analysis of total metal content (by digestion in Parr bombs as described below) of centrifuged and uncentrifuged sediment for two sites. These results for sites 4 and LS are presented in Table 9. It is observed that the upper layer of the centrifuged sample has total metal concentrations about two times higher than those found in the bulk uncentrifuged sample.

Analysis for Total Metals in Sediments

Separate sediment samples from each of the sites were digested in Parr bombs with aqua regia and HF for total metal content determinations (Bernas, 1968). These sediment samples were not centrifuged but were sieved through a #10 mesh stainless steel sieve to remove coarse particles and debris from the sediments. These analyses were conducted to allow comparisons to the EPA pollution status classifications (Appendix D) currently in use for Great Lakes harbors (EPA, 1977). These results are listed in Table 10.

Pesticide and PCB Analysis

Sediment samples were analyzed for selected chlorinated pesticides and polychlorinated biphenyls (PCBs). These chemical compounds affect sediment quality since they are potentially available to biota in the ecosystem. Many

TABLE 9

Total Metal Concentrations in Sediment Samples (mg/kg)-1977

	As	Cd	Cr	Co	Cu	Fe	Pb	Mn	Ni	Se	Zn
$^{\mu}$Site 4	37	<10	<31	43	39	46,900	92	670	65	<4.1	310
$^{\mu}$Site 4	36	<11	<32	34	40	46,800	96	700	49	<4.3	320
$^{\mu}$Site LS	29	<10	<31	44	17	35,600	<31	560	67	<4.2	82
$^{\mu}$Site LS	36	<13	<38	41	20	35,600	<38	590	<46	<5.1	76
^{c}Site 4	38	<24	<70	<75	75	70,800	212	1040	<85	<9.4	550
^{c}Site 4	30	<25	<76	<81	66	75,800	1270	1010	207	<10.1	520
^{c}Site LS	53	<16	<47	94	69	68,800	<47	1090	72	<6.3	178
^{c}Site LS	83	<15	<46	80	67	67,500	<46	1100	71	<6.1	178

$^{\mu}$Uncentrifuged Samples

^{c}Centrifuged Samples

TABLE 10

Total Metal Concentrations in Sediment Samples (mg/kg) - Uncentrifuged Samples-1977

	As	Cd	Cr	Co	Cu	Fe	Pb	Mn	Ni	Se	Zn
Site 1	--	<12	41	50	24	52,600	60	980	180	<4.8	151
Site 1	--	<10	67	33	40	48,100	52	900	155	<4.2	155
Site 3	64	<13	<38	<41	13	38,500	90	360	<46	<5.1	202
Site 3	66	<12	<35	<38	12	37,600	82	490	61	4.7	190
Site 4	28	<15	<46	80	31	49,500	77	710	105	<6.2	275
Site 4	31	<16	<47	66	31	50,000	109	720	56	<6.3	278
Site 5a	38	<12	<35	38	<12	25,900	59	380	42	<4.7	111
Site 5a	19	<12	<36	39	<12	26,700	61	390	<44	<4.9	114
Site 6	28	<13	<38	66	13	40,800	64	590	148	<5.1	214
Site 6	25	<12	<37	51	12	41,700	86	560	142	<4.9	199
Site 6R	97	<10	<30	43	20	42,500	91	550	85	<4.0	221
Site 6R	27	<11	<33	<36	18	42,400	78	540	165	<4.5	214
Site LS	9	<11	<34	<36	18	36,200	57	520	95	<4.5	88
Site LS	39	<11	<33	35	22	39,500	55	550	57	<4.4	88

chlorinated hydrocarbon type organic compounds concentrate in the fatty tissue of aquatic animals according to their solubility characteristics.

Sediment Extraction Procedure --

The extraction of pesticides and PCBs from sediment samples generally followed published procedures (Breidenbach et al., 1964; Bellar and Lichtenberg, 1975). Each sediment was air dried (about 3 days) and a 40 g subsample was mixed with 4 ml distilled water and transferred to a pre-extracted cellulose soxhlet extraction thimble. The sediment was extracted with a 9:1 mixture of hexane-acetone for 8 hours in a soxhlet extraction system. The extract was passed through a column of Na_2SO_4 and concentrated to about 5 ml using a Kuderna-Danish evaporator system equipped with a three ball Snyder reflux column.

Extract Clean-up Procedure --

For removal of lipids, waxes and high molecular weight colored compounds, the extract was passed through a column containing a lower layer of 3% deactivated florisil and an upper layer of Na_2SO_4. Elution was done with a 70:30 mixture of petroleum ether and methylene chloride. The eluent was concentrated to 5 ml using the Kuderna-Danish apparatus. Recovery efficiency was determined to be 86% for PCBs and about 100% for DDT.

The concentrated eluent was subjected to further clean-up for removal of high molecular weight interferences and elemental sulfur using gel permeation chromatography (Kuehl and Leonard, 1978). The extract was injected into the system and pumped (FMI metering pump) through two 2.5 cm diameter glass columns containing a total of about 60 g of SX-2 Bio-Beads (BIO-RAD Laboratories). Methylene chloride was used as the mobile phase solvent. The U.V. absorbance of the eluent was monitored at 254 nm. A standard mixture of corn oil and PCBs was used to determine the point of separation between the high and low molecular weight eluents. Elemental sulfur eluted at a retention volume greater than found for PCBs (and other compounds under analysis) and this late eluting fraction was discarded. The eluent fraction containing the low molecular components was concentrated to 10 ml using a gentle stream of pre-purified nitrogen blown over the surface.

Separation of PCBs and Pesticides --

The separation of PCBs from chlorinated pesticides in the gel permeation cleaned-up extract was accomplished using column chromatography with silica gel as the stationary phase (Snyder and Reinert, 1971). Silica gel was heated at 210°C overnight and covered with pentane. A portion of the silica gel saturated with pentane (5 ml) was transferred to a 10 ml graduated pipet filled with pentane. After rinsing the silica gel column with pentane, 2 ml of the gel permeation cleaned-up extract was placed on the column and eluted with 70 ml of pentane. This eluent contained the majority of the PCB components. Benzene (40 ml) was passed through the column to obtain chlorinated pesticides and the remaining PCB compounds. Spiked samples carried through this procedure showed 84% of the PCBs were contained in the pentane fraction and 100% of the DDT was present in the benzene fraction.

Gas Chromatographic Analysis --

The pentane and benzene fractions from the silica gel separation procedure were concentrated by applying a stream of pre-purified nitrogen over the liquid surface.

General gas chromatographic analysis procedures followed EPA methods (Thompson, 1977). The samples were analyzed using a Tracor MT-220 gas chromatograph equipped with ^{63}Ni electron capture detectors. Two columns were used in the identification and quantitation process. One column contained 4% SE-301/6% OV-210 on Chrom W (HP) 80/100 mesh while the second column was packed with 1.5% OV-17/1.95% OV-210 on Chrom W (HP) 80/100 mesh (Analabs Co.).

The gas chromatograph elution patterns were analyzed for the presence of lindane, aldrin, chlordane, pp'-DDD, pp'-DDE, op'-DDT, pp'-DDT, dieldrin, endrin, heptachlor, heptachlor epoxide, hexachlorobenzene, methoxychlor, oxychlordane, and PCB Aroclor mixtures 1019, 1221, 1232, 1242, 1248, 1254 and 1260. The details of the elution pattern analysis for PCBs are given in Appendix B. The results of these chemical analyses are given in Tables 3 (for 1978) and 11 (for 1977).

The concentrations of lindane, heptachlor, aldrin, heptachlor epoxide, dieldrin and the DDT complex were less than 1.0 μg/kg in all the sediment samples. The total PCB values ranged from about 0.3 to 2.1 mg/kg. For the 1977 summer sediment samples, the chromatographic elution patterns showed best fits with Aroclor 1242 and Aroclor 1254 mixtures. Low levels of p,p'-DDE were found in two of the sediment samples.

Chlorinated Phenol Analysis

Chlorinated phenols are present in certain industrial effluents such as paper processing wastes. Pentachlorophenol has a variety of uses among which include its function as a wood preservative. In order to determine if sediments in the Duluth-Superior harbor were contaminated with pentachlorophenol, an analytical survey was conducted.

Analysis Procedure --

About 100 g of dried sediment was placed in a glass bottle and 500 ml of 0.1 M NaOH solution added. The suspension was shaken for 0.5 hr and centrifuged for 15 minutes at 10,000 rpm. The liquid was decanted into a separatory funnel and extracted with 100 ml of 85:15 hexane-methylene chloride. The organic layer was discarded. The aqueous layer was acidified to pH = 2 with 6M HCl and extracted with two 50 ml portions of 85:15 hexane-methylene chloride. After a final extraction with 50 ml of hexane, the

TABLE 11

PCBs, Pesticides and Pentachlorophenol in Sediments - 1977*

		Silica Gel Samples				
Site**	Total PCBs†	Total PCBs‡	1254#	1242#	p,p' DDE	Pentachlorophenol
1	1.2	1.4	0.42	0.36	<2	1.4
2	0.54	Not Separated			<2	1.0
3	1.4	1.6	0.72	0.24	<2	2.7
4-S	1.9	2.1	0.73	0.39	<2	15
4-F	0.9	0.89	0.31	0.19	9.0	
5a-S	0.72	0.48	0.15	0.07	<2	36
5a-F	0.93	1.0	0.23	0.34	<2	
6	1.7	1.4	0.4	0.37	<2	4.3
6R	0.98	1.1	0.31	0.33	3.1	10
LS-S	0.66	0.49	0.35	0.12	<2	1.2
LS-F	0.38	0.12	No Pattern Discernable			

*PCB values given in mg/kg dry weight of sediment; values are not corrected for spike recovery adjustments (65% for total procedure). DDE and pentachlorophenol concentrations are ng/g dry weight of sediment.

**S indicates sample from summer collection period.
F indicates sample from special fall collection period.

†These values were determined from the sample extracts after florisil and gel permeation chromatography clean-up steps by calculating the concentrations for each PCB component and summing the values for all the components to give the total concentration.

‡Total values were determined from the combination of the PCB values in the pentane and benzene fractions after silica gel separation. Total concentration calculations were determined with the same method used in #3 above.

#The 1254 and 1242 values were determined with the aid of a computer program to be the best fit with the possible PCB standards and the sediment extracts. (Appendix B) combined organic layers were filtered through anhydrous Na_2SO_4 and reduced to about 10 ml in a Kuderna-Danish evaporator system.

A portion of the sediment extract was treated with diazoethane as an ethylating reagent. The diazoethane was generated from N-ethyl-N'-nitro-N-nitrosoguanidine in an aqueous NaOH solution-hexane mixture. The extract was treated with the hexane saturated with diazoethane. Upon completion of the reaction, a solution of 80:20 methanol-H_2O was added to remove by-products of the reaction. The hexane layer was separated and concentrated with a stream of N_2 (Rogers and Keith, 1976).

The concentrated hexane extract was analyzed by gas chromatography using the same equipment as described for pesticide and PCB analysis. The results of this analysis is included in Table 11.

Pentachlorophenol was detected in most of the 1977 sediment samples. However the concentrations were low being in the range of 1 to 15 μg/kg.

PAH Analysis

Polycyclic aromatic hydrocarbons (PAH) can enter ecosystems from fossil fuels and the combusion of fossil fuels. A number of compounds under the general PAH classification are carcinogenic (Panel on Polycyclic Organic Matter, 1972). The sediments were examined to assess possible contamination from PAH's.

Although no information exists on the occurrence of specific PAH compounds in sediments from the Duluth-Superior harbor or Lake Superior, several PAH's have been reported in Great Lakes water or sediment (Strosher and Hodgson, 1975). The compounds, 2-methylanthracene, 9-methylanthracene, benz[a]anthracene, benzo[a]-pyrene, and 3-methylcholanthrene, which contain from three to five aromatic rings, were used in technique development, including the determination of extraction efficiency and analytical sensitivity. Five additional compounds (phenanthrene, 1-methylphenanthrene, perylene, benzo[g,h,i]perylene and dibenz[a,h]anthracene) reported from water or sediment samples (Strosher and Hodgson, 1975; Acheson *et al.*, 1976) were also included as targets in the initial sediment screening procedure. In the quantitation stage of the analysis, an investigation was also made of the occurrence of fluoranthene, pyrene and anthracene in the sediments.

Analysis Procedure--

Portions of the extracts (hexane-acetone extraction, florisil and gel permeation clean-up, and silica gel fractionation) used in the PCB analysis were investigated for PAH compounds. For preliminary screening, the extracts were chromatographed on a SE-30 column using a Varian Aerograph HY-FI Model 600D gas chromatograph with a flame ionization detector. The instrument was operated isothermally at 185-190°C for elution of 2-methylanthracene, 9-methylanthracene, phenanthrene, and 1-methylphenanthrene and isothermally at 265-270°C for elution of benz[a]anthracene, benzo[a]pyrene, 3-methylcholanthrene, perylene, benzo[g,h,i]-perylene and dibenz[a,h]anthracene. Red clay soil was spiked with five PAH compounds and tested for extraction recoveries. The results showed recoveries of 52.4% for 3-methylcholanthrene, 72.5% for benzo[a]pyrene, 66.0% for benz[a]anthracene, 76.8% for 9-methylanthracene and 90.2% for 2-methylanthracene.

None of the PAH compounds under investigation were observed by preliminary screening in the sediment extracts from sites 1, 2 and 3 (Superior Harbor area) or Lake Superior. Elution peaks were noted in the extracts (benzene fraction from silica gel separation) from sites 4 (Superior Harbor), 5, 6 (Duluth Harbor area) and Pokegama Bay.

Further work on identification, and quantitation of compounds was performed at the Environmental Research Laboratory, Duluth. The sediment extracts from sites 4, 6, Pokegama Bay and Lake Superior were analyzed by combined gas chromatograph-mass spectrometry methods (GC/MS). The procedure involved separation of sample components using a Finnigan 9610 GC equipped with a flame ionization detector. Compound separation took place on a column containing 3% OV-101 on Gas-Chrom Q in an oven programmed from 100°C (1 minute) increasing by 4°C/min to a final holding temperature of 225°C. A more detailed description is provided in Appendix B.

Analytical Results--

Several PAH compounds were identified by mass spectrometry in the extracts from sites 4, 6 and Pokegama Bay. GC/MS retention times, molecular weights, formulae, and approximate concentrations for the identified compounds are given in Tables 12, 13 and 14. When more than one compound is listed at a given molecular weight, the order of listing is in decreasing order of identity probability. The determination of identity possibility and the order of probability was determined from a computerized library search of approximately 25,000 compounds (Heller and Milne, 1978). Gas chromatograms for the sediment extracts from the three sites and Lake Superior are given in Appendix B.

PAH's identified from the GC/MS analysis of the sediment extract from site 6 were basically the same as those from site 4, with the exception of the compound that produced the second or later eluting signal at MW 252 from site 4 being absent in site 6. Retention times between compounds of the same MW's at the two sites are in good agreement.

C_4 phenanthrene was identified by GC/MS from the Pokegama Bay sediment extract. The mass spectral library search listed 2,4,5,7-tetramethylphenanthrene, 3,4,5,6-tetramethylphenanthrene, and 1-methyl-7-(1-methylethyl)-phenanthrene as spectra matches with the unknown at MW 234. The compound at MW 234 produced the greatest ion intensity (870) of the PAH compounds. An unknown compound of MW 168 or 169 with a long retention time (47:34 min.) was also present in Pokegama Bay sediment. The library search listed 1,1'-biphenyl-4-amine, 1,1'-biphenyl-3-amine, and 1,1'-biphenyl, 3-methyl as the three possibilities based on similarities of mass spectra. Of these three, 1,1'-biphenyl, 3 methyl was least likely from

Table 12. PAH Compounds from Site 4 Sediment

Retention time (min.)	MW	Formula	Compound	Approximate Concentration* (ppm, dry wt.)
11:30	178	$C_{14}H_{10}$	phenanthrene anthracene	0.72**
13:26	192	$C_{15}H_{12}$	methylphenanthrene	0.32
15:52	202	$C_{16}H_{10}$	fluoranthene	1.24
16:32	202	$C_{16}H_{10}$	pyrene	0.81
18:24	234	$C_{18}H_{18}$	C_4-phenanthrene	---
21:16	228	$C_{18}H_{12}$	benz[a]anthracene triphenylene chrysene	0.97 --- ---
26:00	252	$C_{20}H_{12}$	benzo[k]fluoranthene benzo[e]pyrene perylene	--- --- 0.38
27:24	252	$C_{20}H_{12}$	benzo[a]pyrene benz[e]acephenanthrylene benzo[k]fluoranthene	0.81 --- ---

*Concentrations are not corrected for recoveries through analytical process.

**Where more than one compound is listed at a given MW, concentrations are calculated based on the compound indicated.

Table 13. PAH Compounds from Site 6 Sediment

Retention time (min.)	MW	Formula	Compound	Approximate Concentration* (ppm, dry wt.)
11:28	178	$C_{14}H_{10}$	phenanthrene	0.72**
			anthracene	
13:48	192	$C_{15}H_{12}$	methylphenanthrene	0.27
15:50	202	$C_{16}H_{10}$	fluoranthene	1.27
16:34	202	$C_{16}H_{10}$	pyrene	0.85
18:20	234	$C_{18}H_{18}$	C_4-phenanthrene	---
21:16	228	$C_{18}H_{12}$	benz[a]anthracene	1.06
			chrysene	---
			triphenylene	---
26:02	252	$C_{20}H_{12}$	benzo[k]fluoranthene	---
			benz[e]acephenanthrylene	---
			benzo[j]fluoranthene	---

*,**Same as in Table 12.

Table 14. PAH Compounds from Pokegama Bay Sediment

Retention time (min.)	MW	Formula	Compound	Approximate Concentration* (ppm, dry wt.)
11:24	178	$C_{14}H_{10}$	phenanthrene**	<0.06
18:26	234	$C_{18}H_{18}$	C_4-phenanthrene	---
21:18	228	$C_{18}H_{12}$	benz[a]anthracene*	<0.03
47:34	169	$C_{12}H_{11}N$	unknown	---

*Concentrations are not corrected for recoveries through analytical process.

**Due to weak ion intensity signals, library searches were not used; tentative identifications are made from molecular weights and retention times.

examination of the spectra, as there was no evidence of methyl group loss. The long retention time of this unknown would indicate a high boiling point, possibly higher than the biphenyl-amines

Weak ion intensity signals at MWs 178 and 228 at retention times of 11:24 and 21:18 indicated the presence of phenanthrene (anthracene) and benz[a]anthracene (chrysene, triphenylene), respectively. No methylphenanthrene, fluoranthene, pyrene, or heavier PAH's were identified from Pokegama Bay as they were from sites 4 and 6.

Discussion of Sediment Results

General Sediment Parameters --

The general sediment parameters given in Tables 2 and 3 indicate high levels of certain constituents. COD values were above the 80,000 mg/kg level for sites 1, 3, 4, 6 and Pokegama Bay. A value above 80,000 is in the heavily polluted category according to current Great Lakes harbor criteria (see Appendix D). Ammonia and TKN values were high for sites 3, 4, 5a, 6 and LS. The TKN values classify these sediments as heavily polluted for this parameter. These same sites exhibited elevated levels of oil and grease.

The sediments all showed positive redox potentials except for one sample (site 6-1978). These Eh values were measured on core samples at a distance of about 2 cm below the sediment surface. Mercury levels were below the 1.0 mg/kg standard for all the sediment samples and thus they would be classified as non-polluted with respect to this parameter.

A general consideration of all of these sediment parameters would indicate that site 2 was the least polluted with sites 1 and LS showing low levels of some values (see Table 45, Section 10).

The relationship of these general sediment parameters to potential adverse ecological effects due to their disturbance and disposal is not well understood. For example, a COD value will include a measurement of all material which can be oxidized by a fairly strong chemical oxidizing agent under rigorous conditions. Consequently ammonia and iron (II) would be measured in addition to oxidizable organic matter. The rate and degree of oxygen demand by sediments can vary greatly for the same COD values depending on the species contributing to the COD's.

The use of oil and grease values in determining sediment quality have been questioned (DiSalvo, et al., 1977). The oil and grease value can contain numerous natural and petroleum derived hydrocarbons in addition to fats, oil, waxes, and sulfur. Sediments with high oil and grease values showed low toxicity toward mussels, crabs, clams and snails in their tests.

Metals in the Sediments --

Total metal concentrations in Table 10 indicate elevated values for certain metals in the Duluth-Superior harbor sediments. The results show high levels of lead, iron, nickel and arsenic and moderate to high levels of manganese and zinc in the harbor sediments when compared to classifications of sediment metal levels (Appendix D). These total metal concentrations were obtained by completely dissolving the sediment samples in Parr bombs with aqua regia and HF. Thus they are expected to be higher than those obtained by using a concentrated HNO_3, 30% H_2O_2 digestion procedure (Fuller, 1969). This latter procedure gives metal concentrations in the sediments compatible with values listed in EPA guidelines but does not include metals within the silicate lattices in the particles. Consequently our total metal values cannot be directly compared to EPA guideline values.

The classification of the sediment metals according to their release characteristics to various chemical extractants provides information on the nature of their bioavailability (Tables 3 through 8). Within the detection limits of the analyses, only iron and manganese were extracted from the sediments in the exchangeable and easily reducible phases. However, many metals were associated with the organic and sulfide phase of the sediment. As shown in Table 6, detectable amounts of arsenic, cobalt, copper, nickel and zinc were found in the organic and sulfide phases as were selenium and cadmium in some of the samples. The metals in this phase are potentially available to biota in the ecosystem (Engler et al., 1974). The amounts of nickel and zinc in some of the samples were greater in the organic and sulfide phases than in any of the other phases.

The metal concentrations in the moderately reducible phase of the sediments were generally lower than in the organic and sulfide phases. However, cobalt and nickel showed similar concentrations to those found in the organic and sulfide phase. Detectable amounts of arsenic, copper and zinc were found in the moderately reducible phase and large amounts of iron were present.

The residual phase of the sediments contained detectable levels of all investigated metals. Compared to the other phases, arsenic, cobalt, iron, nickel and selenium were found in the highest amounts in the residual phase. The amounts of copper and zinc were similar to that found in the organic and sulfide phase. The amount of lead in the residual phase was less than determined in the organic and sulfide phase. Manganese was distributed throughout the separate phases with the largest amounts detected in the residual and organic and sulfide phases of the sediments.

Excluding the metals in the residual phase of the sediments and summing the concentrations of metals in the other phases indicates that the non-residual (and potentially bioavailable) metal levels were generally not high. All of these values would fall below the heavily polluted category with the exception of nickel for four sites (particularly site 6 which may have been contaminated) and lead for three sites.

Trace Organics in the Sediments --

The concentrations of PCBs in the sediment samples were not high. Tables 3 and 11 indicate a total PCB range of 0.3 to 2.1 mg/kg. The samples obtained at the same site during two different years show good agreement.

The values determined in this study are higher by about a factor of ten compared to results from a recent Corps of Engineers harbor survey (Whiting, 1977 and are below the 10 mg/kg upper limit for unpolluted sediments (Appendix D).

The sediment data indicate a general absence of chlorinated hydrocarbon pesticide compounds in the sediments. The concentrations of pentachlorophenol were low (1 to 36 ng/g).

The concentrations of the PAH compounds identified in sediments from two sites (4 and 6) were about 1 mg/kg or less. There are no sediment quality standards for these compounds. At these low levels, it is very unlikely that any adverse ecological effects would result from their presence in the sediments. Of the compounds tentatively identified, benz[a]anthracene is carcinogenic and benzo[a]pyrene is highly carcinogenic.

OVERLYING WATER

Samples of water overlying the sediments were obtained by siphoning a portion of the water contained in the core liners. These samples were analyzed for a number of physical and chemical parameters indicative of overall water quality. Specific procedures used in the analysis are given in Appendix A and the results are presented in Table 15. In addition to these data, measurements of dissolved oxygen, temperature and specific conductance were made in the field during sampling. The results of these field measurements are given in Appendix C.

The water samples were not filtered and consequently the results do include contributions from suspended particulate matter. It is likely that the metals are largely associated with fine particles.

There are generally no large differences in parameters between sites. Site 2 overlying water is seen to be particularly low in nitrogen values. The Duluth harbor water exhibits higher specific conductance values than found for Superior harbor water (Appendix C).

TABLE 15

CHEMISTRY OF WATER OVERLYING SEDIMENT - 1977

	SITE							
Parameter*	1	2	3	4	5a	6	6R	LS
pH	7.5	6.7	6.3	6.8	7.2	---	7.0	7.5
Total PCBs	0.04	---	0.2	0.04	0.03	0.2		
H_2S	0.17	0.20	0.20	<0.17 <0.19	0.19 0.20	---	<0.17 <0.18	<0.17 <0.17
NH_3	0.90	0.05	3.7 3.4	4.3	4.2	2.2	3.8	1.6
TKN	7.0	0.05	5.9 7.8	10.5	12	9.7	16	14.5
COD	36.5	33.2 32.2	60.1 60.1	64.2 50.1	46.7 54.9	72.5 85.9	58.5 47.5	14.3 15.4
PO_4	0.085	---	0.057	0.320 0.290	0.088 0.088	0.180	0.670 0.660	0.040 0.046
Suspended Solids	92	42 45	211 221	25 20	27 44	22 22	124 127	46 72
Chloride	9.3	10.2 10.1	7.8 8.2	17.7 14.7	10.1 10.7	14.2 14.2	12.8 13.4	6.4 6.1
Organic N	6.1	<0.05	2.5 4.1	6.2	7.8	7.5	12.2	12.9
Arsenic	2.4	2.1	6.0	3.0	1.9	2.2	3.3	1.7
Cadmium	<0.05	0.67	0.10	<0.05	<0.05	<0.05	<0.05	<0.05
Chromium	<0.2	<0.2	2.5	1.4	0.4	0.4	<0.2	<0.2

TABLE 15 (continued)

Parameter*	1	2	3	4	5a	6	6R	LS
Cobalt	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Copper	5.6	2.1	17	53	60	97	25	17
Iron	450	310	570	290	230	430	670	230
Lead	8.1	3.4	2.4	1.7	1.3	2.2	1.0	<0.1
Manganese	31	24	110	48	58	170	51	4
Nickel	3.6	<2	<2	2.3	<2	2.3	<2	<2
Selenium	2.1	2.1	1.5	1.5	1.8	2.1	1.3	1.5
Zinc	3.2	6.5	20	5.0	5.2	6.5	2.5	1.4
Inorganic Mercury	<0.05	0.08	<0.05	<0.05	<0.05	0.07	<0.05	<0.05
Organic Mercury	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

*Values in mg/l except for PCB and metal concentrations in µg/l.

INTERSTITIAL WATER

The interstitial water was obtained by high speed centrifugation under a nitrogen atmosphere and stored in teflon bottles for chemical analysis and bioassay tests. This procedure was described at the beginning of SECTION 7. Analysis procedures are given in Appendix A and the results in Tables 16 and 17.

Portions of the interstitial water were used in 1977 bioassay tests (SECTION 8). The water was not filtered in order to preserve its characteristics as nearly as possible to the water which might be released to the water column upon dredging or be present in the sediment for exposure to burrowing aquatic animals. The chemical analyses were performed on the unfiltered water to characterize the systems used in the bioassay tests. It is likely that many of the chemical species (such as metals) are associated with very fine (possibly colloidal) particles in the water (Leckie and James, 1974). In addition, significant portions may be complexed or adsorbed to organic or inorganic species. Complexation of metals can greatly reduce their toxicity characteristics as compared to the dissolved aquo forms (Andrew, *et al*. 1977).

The variation in metal content between filtered and unfiltered samples is shown in Table 17. The arsenic, cadmium, chromium, copper, iron, lead and zinc concentrations in the filtered samples were generally much less than found for the unfiltered samples. Only the manganese concentrations did not change much upon filtering the samples (0.45 micron pore).

For most of the metals investigated, there were no large differences between interstitial waters of the harbor sediments and the Lake Superior sediment (LS). However, unfiltered interstitial water from the Lake Superior site sediment was generally lower in iron, manganese and zinc than unfiltered interstitial water from the harbor sediments.

TABLE 16

CHEMISTRY OF INTERSTITIAL WATERS - 1977

	SITE								
Parameter*	1	2	3	4	5a	6	6R	LS	Pokegama
pH	7.1	7.2	6.0	---	---	7.1	6.7	7.1	---
COD	50.6 52.9	285 99.4	175 171	115 119	77.7 78.1	146 145	208 224	70.6 71.7	93.6 89.9
H_2S	<0.32	<0.20	<0.17	<0.17	0.19	.44	0.20	<0.17	<0.17
Cl	61.4 62.5	8.9 7.9	21.4 21.9	12.6 13.0	13.3 14.6	24.0 23.8	18.5 18.4	4.18 4.11	12.1 11.9
NH_3	2.6	11.7	21	15.5	11	3.8	20	16	5
TKN	3.7	13.3	21	30	22	7.6	46	16.5	17
Total PCBs	0.2	0.5	1.4	3.5	0.4	0.7	---	---	3.1
Organic N	1.1	1.63	0	14.5	11	3.8	26	0.5	12
Suspended Solids	35	45 40	51	83	66	45	118	41	36 27
Arsenic	5.0	4.1	3.8	3.8	2.8	6.0	5.5	6.1	10
Cadmium	<0.05	0.32	2.0	0.05	<0.05	0.30	0.10	2.0	1.9
Chromium	0.5	0.5	7.1	4.4	1.1	7.1	3.7	1.5	12
Cobalt	1.3	3.9	<0.5	0.7	<0.5	1.2	<0.5	<0.5	0.7
Copper	11	11	153	24	12	36	15	60	10
Iron	13100	570	8100	8000	1700	8200	21400	1400	8100
Lead	0.9	0.9	23	4	3	13	12	6	4
Manganese	6000	6000	960	2450	1400	2300	3200	540	1000
Nickel	<2	<2	9.5	2.3	<2	2.3	<2	7.8	3.6

TABLE 16 (continued)

Parameter	1	2	3	4	5a	6	6R	LS	Pokegama
Selenium	1	4	1	2	2	2	1	4	4
Zinc	47	10	68	10	7	14	10	10	10
Total Mercury	0.05	1.7	1.1	0.86	0.32	0.42	0.23	0.44	0.15

*Values listed in mg/l except for PCBs and metals which are given in µg/l.

TABLE 17

METAL CONTENT OF INTERSTITIAL WATERS* - 1978

Metal	SITE 1 U	 1 F	 2 U	 2 F	 3 U	 3 F	 4 U	 4 F	 4a U	 4a F	 6 U	 6 F	 LS U	 LS F
Arsenic	3.3 ±0.5	<1.0	7.1 ±1.3	<1.0	3.2 ±1.5	<1.0	1.3 ±1.0	<1.0	---	---	5.3 ±2.8	3.8 ±2.9	6.4 ±1.9	4.7 ±0.4
Cadmium	0.77 ±0.5	<0.05	0.47 ±0.12	0.09 ±0.03	0.63 ±0.06	<0.05	0.4 ±0.07	0.10 ±0.10	1.3 ±0.2	0.16 ±0.04	0.55 ±0.37	0.41 ±0.05	1.5 ±0.1	---
Chromium	2.2 ±0.3	<1.0	2.5 ±0.1	<1.0	6.7 ±0.6	1.7 ±0.3	5.1 ±1.3	<1.0	4.4 ±0.7	1.5 ±0.2	6.0 ±5.9	1.5 ±0.5	<1.0	<1.0
Cobalt	<1.0	---	<1.0	1.0 ±1.0	1.5 ±0.2	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Copper	18.7 ±3.4	2.0 ±1.4	27.7 ±12	4.8 ±0.5	27.2 ±4.7	2.5 ±0.5	17.8 ±7.7	1.5 ±0.4	69 ±16	6.7 ±0.2	92 ±21	56 ±29	26.3 ±8.9	18.9 ±6.5
Iron	5.0 ±0.8	0.49 ±0.08	17.9 ±9	0.35 ±0.18	24.7 ±2.8	18.2 ±3.7	23.5 ±4.2	12.7 ±4.8	15.2 ±5.2	1.2	10.2 ±2.4	7.4 ±1.8	3.2 ±0.7	0.32 ±0.15
Lead	14.2 ±3.6	1.5 ±1.4	8.2 ±5.1	---	22.7 ±3.8	1.0 ±0.2	10.3 ±4.4	0.3 ±0.2	35 ±14	0.7 ±0.7	5.5 ±2.9	3.2 ±1.0	5.9 ±0.8	3.4 ±1.1
Manganese	1.30 ±0.03	1.06 ±0.02	3.9 ±0.5	3.0 ±0.6	1.81 ±0.03	1.76 ±0.16	1.89 ±0.41	1.65 ±0.37	1.61 ±0.02	1.16 ±0.04	1.98 ±1.02	1.87 ±1.0	1.61 ±0.06	1.51 ±0.06
Nickel	1.1 ±1.1	<1.0	<1.0	<1.0	2.7 ±1.1	<1.0	2.4 ±0.9	1.4 ±1.2	---	<1.0	1.3 ±1.4	1.2 ±0.7	3.7 ±0.5	3.5 ±1.8
Zinc	42 ±44	---	16 ±12	13 ±12	34 ±2	5.4 ±2.2	27 ±15	4.3 ±0.5	31 ±1	8.8 ±4.9	20 ±6	23 ±8	5.2 ±0.3	---

---

*Concentrations are in μg/l except for iron and manganese which are listed in mg/l. The uncertainties in the values are standard deviations based on triplicate analyses at each site.

Compared to the overlying water, the unfiltered interstitial water was generally greatly enriched in iron (by factors of 15 to 100), manganese (by factors of 10 to over 100) and mercury (by factors of 5 to 20) even though the concentrations of suspended solids were about the same. In addition, the interstitial water generally contained higher amounts of arsenic and about two to ten times as much chromium and zinc. Even in the filtered interstitial water samples, iron and manganese concentrations were generally 2 to 100 times greater than in the overlying water.

A comparison of the sites generally does not show large variations in parameters for the 1977 sampling. However site 3 sediment interstitial water contained the highest amounts of copper (153 μg/l), lead (23 μg/l), nickel (9.5 μg/l) and zinc (68 μg/l). Variations between sites 6 and 6R were large for COD, nitrogen, and some of the metals. This variation may be due, in part, to the higher level of suspended solids in the intersitial water for sample 6R.

ELUTRIATE WATER

The procedures for evaluating dredged material for potential disposal effects include an investigation of solid, liquid and particulate phases resulting from the mixing of one volume of sediment with four volumes of water (EPA/Corps of Engineers, 1977). The water phase obtained after the mixing process has been called elutriate water (Keeley and Enger, 1974). The elutriate test has been evaluated for its appropriateness in predicting the pollution characteristics of dredge material (Lee, et al., 1975; Cheam, et al., 1976).

In this study, liquid and particulate phase systems were used in certain of the acute toxicity bioassay tests (SECTION 8). The liquid phase obtained by mixing test sediments with Lake Superior water was characterized by analysis of an array of physical and chemical parameters.

The procedure used in preparation of the liquid and particulate phase systems generally followed the EPA/Corps of Engineers guidelines (1977).

One variation in proceudre involved elimination of the filtering step for obtaining the liquid phase. It was replaced by using high speed centrifugation to remove about 99% or more of the suspended material from the particulate phase. Filtering was omitted since it would be lengthy and risk causing changes in the liquid phase through such processes as loss of volatiles, oxidation of components and loss of organics and metals on the filter and retained solids.

A second variation involved using two sediment samples from cores from each of the 1977 sites in preparing the liquid phase. One sediment sample was exposed to air and the liquid phase resulting from mixing the sediment with Lake Superior water is called oxic elutriate water. A second sediment sample from each site was not exposed to air but the sediment-water suspension was produced under a nitrogen atmosphere. The liquid phase resulting from the mixing of the unexposed sediment with Lake Superior water is called anoxic elutriate water. The Lake Superior water was well-oxygenated in both cases. Consequently, the terms oxic and anoxic are used here only to designate whether or not the sediment had been exposed to air.

The two sediment samples were used to investigate possible chemical releases and potential toxic effects resulting at the dredging site (releases from sediment not exposed to air) and at the disposal site (sediment exposed to air).

Preparation of Liquid Phase (Elutriate Water) and Particulate Phase

Sediment from a given sampling site was obtained from the upper portions of several cores. The sediment was added to a 3 l beaker containing about 400 ml of Lake Superior water (obtained from the Environmental Research Laboratory-Duluth, Minnesota) with mixing until the total volume reached 1400 ml. The suspension was transferred to a 12 l round bottom flask and enough Lake Superior water was added to produce 5 l of suspension (1 l of sediment - 4 l of water). The flask was placed on a shaker bath and shaken for 0.5 hr at 100 excursions per minute. The suspension was allowed to settle for 1 hour. (For the 1978 bioassay tests, a portion of the overlying suspension (particulate phase) was removed). The overlying suspension was transferred to 250 ml centrifuge bottles (stainless steel for portions to be analyzed for organics and polycarbonate for portions to be analyzed for metals) and centrifuged for 15 minutes at 10,000 rpm. The supernatant liquid (liquid phase elutriate water) was decanted into teflon bottles for use in subsequent chemical and biological tests. For producing the liquid phase anoxic elutriate water, the mixing of sediment with Lake Superior water was carried out in a glove bag under a nitrogen atmosphere.

The elutriate water used in the 1978 bioassay tests (SECTION 8) was obtained by recentrifuging the supernatant from the first centrifuging at 14,000 rpm for 10 minutes.

Liquid Phase (Elutriate Water) and Particulate Phase Tests

Portions of the liquid phases were used in bioassay tests run in 1977 and 1978. Portions of the particulate phases were used in 1978 bioassay tests (SECTION 8). The liquid phase (formed by use of sediments from the various sites) was analyzed for a number of physical and chemical parameters. General water analysis procedures are given in Appendix A and the analytical results are listed in Tables 18 through 21.

Survey of Chemical Properties of Elutriate Water

A general survey of Tables 18 and 19 shows that the exposure of sediments to air prior to use in elutriate water preparation did not greatly alter the chemical water parameters from values found for sediment unexposed to air. The elutriate waters produced from air exposed sediments had higher ammonia values (by about a factor of two) for most of the sites. However the other chemical parameters do not show any definite trends caused by exposure to air.

Table 20 indicates that the dissolved oxygen concentrations in the liquid phase remained above 3 mg/l in all cases and above 5 mg/l in most

TABLE 18

CHEMISTRY OF ELUTRIATE WATER (ANOXIC SEDIMENT CONDITIONS) - 1977

	SITE								
Parameter*	1	2	3	4	5a	6	6R	LS	Pokegama
pH	6.9	6.9	---	6.0	6.9	5.8	6.4	7.1	---
H_2S	<0.17	0.20	0.20	0.22	<0.17	<0.17	0.20	<0.17	<0.17
Ammonia	5.5	1.8	10.5	13	7	8	12	11	3.0
TKN	5.8	6.8	24.5	19	16	11	26.5	11	5.3
Organic N	0.3	5	14	6	9	3	14	<2	2.3
COD	28.9	241	90.8	101	52.7	111	84.0	41.8	92.1
Total PCBs	0.3	0.2	1.4	2.5	0.9	0.9	---	---	2.6
Arsenic	5.6	7.3	---	6.0	3.4	6.5	6.2	4.3	---
Cadmium	0.20	0.15	---	0.85	0.05	0.10	0.05	0.20	---
Chromium	8.0	1.4	---	15.0	1.8	3.3	4.1	5.9	---
Cobalt	2.7	0.8	---	<0.5	<0.5	0.5	0.8	0.7	---
Copper	35	9	---	37	17	32	18	21	---
Iron	4400	800	---	1900	2100	2100	3600	2900	---
Lead	4.1	1.7	---	11	6.7	3.3	6.0	1.7	---
Manganese	285	910	---	345	215	350	350	350	---
Nickel	6.4	<2.0	---	<2.0	<2.0	2.3	<2.0	23	---
Selenium	1	2	---	4	2	1	1	2	---
Zinc	21	5.9	---	22	17	18	57	34	---
Mercury	<0.10	0.25	0.16	0.21	<0.10	0.22	<0.10	<0.10	<0.10

*Values listed in mg/l except for PCBs and metals which are given in µg/l.

TABLE 19

CHEMISTRY OF ELUTRIATE WATER (OXIC SEDIMENT CONDITIONS) - 1977

	SITE								
Parameter*	1	2	3	4	5a	6	6R	LS	Pokegama
pH	7.0	7.2	---	6.0	6.9	5.6	6.5	7.4	---
H_2S	<0.17	0.17	0.20	0.18	0.19	0.44	<0.17	<0.17	---
Ammonia	10	6.7	16	22	11	16.5	20	8	3.5
TKN	10	7.2	24.5	24.5	22	17	38	11.5	7.5
Organic N	<2	<2	8.5	2.5	11	<2	18	3.5	4.0
COD	42.7	222	105	82.4	54.3	94.4	116	54.0	88.7
Total PCBs	0.5	0.2	1.2	2.5	0.3	0.9	---	---	1.6
Arsenic	16.5	6.5	---	9.5	5.6	8.4	8.0	7.7	---
Cadmium	---	<0.05	---	0.30	0.05	0.25	0.35	0.20	---
Chromium	9.7	0.3	---	2.5	3.7	7.1	9.0	3.4	---
Cobalt	2.8	<0.5	---	<0.5	<0.5	1.3	0.5	1.6	---
Copper	10	3	---	36	11	15	31	21	---
Iron	4700	400	2800	1600	830	5200	2900	2300	---
Lead	2.9	0.4	---	12	4.0	16	13	1.2	---
Manganese	308	960	---	515	390	525	490	225	---
Nickel	9.3	<2.0	---	2.3	<2.0	2.3	9.7	5.0	---
Selenium	---	2	---	4	2	2	2	1	---
Zinc	28	1.5	---	22	13	26	58	38	---
Mercury	<0.10	<0.10	0.14	<0.10	<0.10	0.41	0.12	<0.10	<0.10

*Values listed in mg/l except for PCBs and metals which are given as µg/l.

TABLE 20

PHYSICAL AND CHEMICAL DATA* FOR LIQUID PHASE ELUTRIATE WATER - 1978

Date	Site	pH	D.O.	COD	NH_3	Specific Conductance	Suspended Solids (Liquid Phase)	Suspended Solids (Particulate Phase)
June 5	1	6.6	5.4	165	0.48	104	105	14,100
June 26	2	6.4	7.6	23	1.3	153	24	5,600
July 15	2	6.5	5.6	31	1.3	110	63	11,800
June 19	3	6.6	3.4	---	3.2	120	101	7,900
June 12	4	6.7	5.6	107	2.7	105	73	9,300
July 15	4a	6.4	5.2	44	1.2	97	119	15,600
June 26	5	6.3	4.9	38	0.5	71	79	10,700
July 10	5	6.3	6.3	43	1.1	71	74	3,200
July 10	6	6.6	4.2	36	0.3	102	141	15,400
June 5	LS	6.6	7.5	109	0.5	100	28	1,900
June 12	LS	6.7	7.8	3	0.5	100	28	4,000
June 19	LS	7.0	8.4	---	0.15	100	60	3,300
June 26	LS	6.2	4.8	14	0.5	68	69	7,600
July 10	LS	6.4	5.3	13	0.2	89	49	5,400
July 17	LS	6.6	7.3	18	2.3	133	25	2,200
July 24	LS	---	---	23	---	---	69	6,700

---

*Units are mg of O_2/l for DO and COD, mg/l for NH_3, µmhos/cm for specific conductance and mg/l for suspended solids. pH, D.O., COD and NH_3 were measured on liquid phase (centrifuged) elutriate.

TABLE 21

METAL CONCENTRATIONS* IN LIQUID PHASE ELUTRIATE WATER - 1978

Date	Site	As		Cd		Cr		Co		Cu		Fe		Pb		Mn		Ni		Zn	
		U	F	U	F	U	F	U	F	U	F	U	F	U	F	U	F	U	F	U	F
June 2	1	9.6	2.6	0.64	0.78	7.2	2.2	2.7	<1.0	24	11.4	5.0	1.15	3.1	1.0	0.36	0.28	8.1	3.9	24	9.3
June 26	2	5.0	1.5	0.05	0.06	1.4	<1.0	<1.0	<1.0	6.5	3.6	0.60	0.32	1.2	0.4	0.75	0.82	<1.0	<1.0	5.2	6.1
July 15	2	3.7	2.9	0.57	0.40	2.7	<1.0	1.1	<1.0	42	9.3	2.4	0.32	3.7	0.4	0.50	0.38	3.1	2.3	13	16
June 17	3	8.0	2.1	0.20	0.05	2.6	<1.0	1.1	<1.0	17	2.6	3.1	0.12	3.7	0.1	0.24	0.18	3.9	<1.0	17	4.2
June 8	4	10.5	3.2	0.20	<0.05	4.5	<1.0	1.1	<1.0	17	2.3	3.7	0.41	4.5	0.2	0.26	0.21	2.0	<1.0	15	4.2
July 15	4a	4.9	1.6	0.23	0.31	5.2	1.2	1.3	<1.0	45	7.8	5.5	0.30	10	0.5	0.28	0.13	7.8	2.0	28	13
June 26	5	9.5	1.0	0.18	0.05	4.6	<1.0	1.2	<1.0	21	1.6	2.8	0.14	4.9	0.3	0.09	0.06	3.0	<1.0	22	5.9
July 6	5	4.9	2.1	0.08	0.45	4.2	<1.0	<1.0	<1.0	20	4.1	2.9	0.69	7.2	0.7	0.16	0.10	3.2	<1.0	25	16
July 24	5	3.9	3.3	0.08	0.21	3.3	<1.0	<1.0	<1.0	20	3.2	2.7	0.06	7.0	0.2	0.14	0.07	2.8	<1.0	24	5.6
July 6	6	15	4.9	0.85	0.08	9.0	1.1	1.0	<1.0	35	7.3	5.8	0.74	19	1.3	0.24	0.16	6.2	<1.0	69	7.5
July 24	6	<1.0	1.4	0.08	0.65	2.6	---	<1.0	<1.0	19	6.8	2.6	0.74	3.3	0.4	0.32	0.24	3.9	2.0	21	15
June 5	LS	7.0	4.7	0.68	0.30	1.7	<1.0	1.0	<1.0	13	4.9	0.86	0.56	1.6	0.7	0.91	0.77	<1.0	<1.0	8	4.6
June 12	LS	6.2	1.6	0.65	0.26	1.8	<1.0	<1.0	<1.0	28	7.5	1.5	0.54	1.3	0.3	0.58	0.58	1.1	<1.0	8	4.2
June 19	LS	9.8	4.9	0.97	0.26	2.4	<1.0	1.0	<1.0	22	9.2	1.5	0.49	2.6	1.2	0.96	0.82	2.0	4.0	9	5.1
July 10	LS	11	6.2	1.2	0.46	2.5	<1.0	1.1	<1.0	17	7.2	2.4	0.74	2.9	0.6	0.50	0.36	3.5	1.1	13	9.5
July 18	LS	9.8	9.2	0.36	0.33	<1.0	1.2	1.0	<1.0	19	11.8	0.74	0.20	3.6	0.1	2.53	2.04	2.0	1.1	7	10
July 24	LS	5.2	2.9	0.88	0.13	1.6	<1.0	1.6	<1.0	26	0.8	2.0	0.48	3.3	<0.1	0.75	0.56	4.4	1.8	11	1.6

*Units for metal concentrations are µg/l except for Mn and Fe which are mg/l. The U and F headings represent unfiltered and filtered (0.45 micron pore) liquid phase elutriate water.

cases. The particulate phase contained about two to fifteen grams per liter of suspended material.

Table 21 indicates the effect of filtering the liquid phase on metal concentrations. Metals concentrations were generally decreased upon filtering. This could result from either adsorption on the filter paper (cellulose acetate) or removal of particulates along with their adsorbed metals. Considering iron and manganese, there was a much greater per cent decrease in the concentrations of iron than manganese. This greater effect of filtration on iron concentrations could indicate the presence of particulate iron in the form of oxides or hydrous oxides in the liquid phase elutriate water which are largely removed from solution upon filtration.

Comparison of the chemical parameters of the Lake Superior site to those for the harbor sites does not generally show large differences. Except for one instance, the LS liquid phase elutriates had lower COD and NH_3 values. The metal concentrations were similar in the elutriate waters (filtered and unfiltered) for the LS samples as compared to harbor site samples. The reason for one particularly high manganese concentration in the LS elutriate water from July 18, 1978 sampling is unknown.

GENERATED PORE WATER

Certain fractions of sediment chemical consituents can potentially be released to the water. This release may be a diffusion process influenced by diagenesis of the minerals in the sediments or it could occur upon disturbing the sediments and mixing them with the overlying water. The released chemical species may be dissolved in the water or associated with fine particulate material which can remain suspended for long periods.

In conjuction with assessing potential harmful effects of dredged sediments, some experiments were carried out to provide information on the rate and amount of release of chemical species from sediments when mixed with "pure" water.

After removal of interstitial water from a given sediment sample, the same volume of Lake Superior water was mixed with the sediment, allowed to remain in contact for a certain period (described below), and removed. This sediment extraction process with Lake Superior water was repeated several times without directly exposing the sediment to air. The Lake Superior water extracts were analyzed for COD, D.O., pH, certain metals and, in some cases, PCBs. From the concentrations of these chemical species in the Lake Superior water extracts and the weights of sediment used, the amounts of chemical species released per kg of sediment were computed. The Lake Superior water extracts are called generated pore water.

Generated Pore Water Production Procedure

Sediment was added to ten weighed stainless steel centrifuge bottles under a nitrogen atmosphere and reweighed. The bottles were centrifuged at 10,000 rpm for 15 minutes using a refrigerated centrifuge (4°C). The centrifuge bottles were placed in a glove bag under a nitrogen atmosphere, the caps were removed and the interstitial water was decanted into teflon bottles and its volume recorded. The caps were replaced and the bottles and sediment weighed. The interstitial water was recentrifuged in polycarbonate centrifuge bottles at 14,000 rpm for 30 minutes and the supernatant was collected for chemical analyses.

The sediment was removed from the stainless steel centrifuge bottles and placed in a blender under nitrogen. A small portion was used to determine the percentage of water in the sediment. A volume of Lake Superior water (equal to the volume of removed interstitial water) was added. The sediment and water were blended at low speed for five minutes and the resulting slurry was placed back into the ten centrifuge bottles and capped. The bottles were refrigerated until the water was to be removed by centrifugation and Lake Superior water again added using the same procedure. The centrifuge bottles and sediment were weighed each time after the removal of the water. After the initial removal of interstitial water and addition of Lake Superior water to the sediment, the process was repeated after 12 hrs, 1.5 days, 3.5 days, 7.5 days, 10.5 days, 15.5 days and 21.5 days.

During one experiment, two stainless steel centrifuge bottles filled with Lake Superior water were carried through the same water removal and water addition process. The water was analyzed for metals to test for leaching from the stainless steel containers.

The procedure described above was completed for sediment from site 4R. In addition, some data was obtained using site 6R sediment but a breakdown in the centrifuge prohibited obtaining data after 10.5 days. An initial experiment was carried out using site 6 sediment but weights were not recorded and only chemical specie concentration values were obtained for the generated pore water.

Some data was also obtained using polycarbonate bottles in place of stainless steel. The effect of blending the sample on concentrations of chemical species in the water was investigated.

Generated Pore Water Results

The results of the initial study of the concentrations of chemical species in the initial interstitial water and generated pore water for site 6 sediment are given in Table 22. Also included is data on the values of these chemical species in Lake Superior water and in Lake Superior water kept in the stainless steel centrifuge bottles (blanks). The values of the chemical parameters in the Lake Superior water and blanks are all low compared to the water obtained by using the site 6 sediment. For the generated pore water, the values of the chemical species tend to increase over those found for the interstitial water (initial values) except for manganese. Significant amounts of the chemical species continue to be extracted even after seven extractions (21.5 day values). These water samples were not filtered and thus the chemical values may include contributions from species

TABLE 22.

INTERSTITIAL AND GENERATED PORE WATER FOR SITE 6*.

						Site 6	Pore Water			Inorganic	Total		
Time	As	Cd	Cr	Cu	Fe	Pb	Mn	Ni	Zn	Hg	Hg	Co	COD
Initial	5.1	0.05	4.6	18.0	9.6	8.7	2400	<2.0	14	0.17	---	<0.5	146.2 145.0
12 hr.	15.8 21.5	1.10 1.45	15.2 18.8	105 77	7.1 7.1	65	1400 1100	14.1 17.1	56	0.24	0.22	0.8	635.9
1½ day	29.4	0.95	19.0	97	10.4	112	1890	10.8	79.2	0.54	0.51	0.6	893.2
3½ day	10.7	1.40	18.9	130	10.6	94	1280	19.3	73.6	0.3	0.2	0.8	834.3
7½ day	10.7	1.05	5.4	28.5	3.8	9.8	1940	6.9	26	0.13	0.38	<0.5	283.9
10½ day	21.2	18.95	8.9	54	3.9	17.1	1840	31.7	80	3.08	3.09	<0.5	323.6
15½ day	8.9	0.40	7.0	36.4	3.5	12.2	1670	10.1	62	0.05	0.42	<0.5	196.1
21½ day	7.2	0.20	7.3	44	2.4	6.0	1890	6.9	25.6	0.26	0.23	<0.5	167.2
						Lake Superior	Blanks**						
12 hr	<0.5	<0.05	1.1	4.9	0.018		11.9	2.1	2.2			<0.5	
1½ day	0.9	<0.05	<0.2	9.5	0.038	0.2	5.3	<2.0	2.3			<0.5	
7½ day	0.6	<0.05	<0.2	32.7	0.012	0.2	17.8	2.3	3.9			<0.5	
10½ day	0.7	<0.05	<0.2	6.6	0.011	0.2	4.6	<2.0	6.3	0.14	0.55	<0.5	
Lake Superior Water	1.1	<0.05	<0.2	0.9	0.013	0.4	1.9	<2.0	2.4			<0.5	

---

* All values are in µg/l except for Fe and COD which are in mg/l. Se was less than one µg/l in all samples.

**Blanks were in stainless steel cups.

associated with fine suspended particulate material not removed during centrifuging.

Experiments with sites 4R and 6R sediments included measurements of the weights of sediments used and this allowed calculation of the amount of chemical releases per kg of dry sediment. Table 23 includes data on sediment weights, volume of water removed from the sediments, pH and dissolved oxygen values. The pH values are seen to remain in the 6.2 to 6.9 range for the extracted water. The dissolved oxygen levels in the water drop as extractions proceed but eventually increase for the latter extractions. The pH of Lake Superior water used in producing the generated pore water was about 7.5 and the dissolved oxygen levels were about 7 to 9 mg/l.

The concentrations of chemical parameters for the interstitial and generated pore water for sites 4R and 6R are given in Tables 24 and 25. Most of the chemical parameters were higher in the generated pore water samples than found for the initial interstitial waters (exceptions are iron and manganese). The values for COD, iron, chromium and lead tend to decrease during the latter extractions. The data for site 4R covers seven sediment extractions while that for site 6R includes five extractions.

Tables 26 and 27 list the total number of mg or μg of the chemical parameters removed from the sediment during the water extraction process. Considering the values for the metals, iron and manganese gave the largest extracted amounts corresponding to the much higher abundances of these metals in the sediments. The amount of release per kg of sediment follows the trend of concentrations in the water extractants. Comparing the two sites, the amounts of extracted chemical parameters are similar.

Discussion of Results

The application of the generated pore water results to the environmental effects of sediment dredging is difficult. However the data may give a

TABLE 23

GENERATED PORE WATER DATA

Site 4R

Time	Wet Weight	Dry Weight	Volume H_2O	pH	D.O.
Initial	1978.53 g	1163.38 g	817 ml	6.40	3.3 ppm
12 hr.	2078.99 g	1222.45 g	518 ml	6.39	1.35 ppm
1½ day	1768.75 g	1040.03 g	450 ml	6.30	0.65 ppm
3½ day	1515.24 g	890.96 g	390 ml	6.25	0.35 ppm
7½ day	1173.63 g	690.09 g	455 ml	6.20	1.20 ppm
10½ day	1012.10 g	595.12 g	430 ml	6.85	2.35 ppm
15½ day	926.87 g	544.99 g	500 ml	6.69	2.60 ppm
21½ day	822.30 g	483.51 g	440 ml	6.60	5.10 ppm

Site 6R

Time	Wet Weight	Dry Weight	Volume H_2O	pH	D.O.
Initial	2631.44 g	1539.39 g	765 ml	6.70	3.10 ppm
12 hr.	1959.77 g	1146.46 g	590 ml	6.70	2.10 ppm
1½ day	1921.86 g	1124.28 g	565 ml	6.71	0.60 ppm
3½ day	1493.69 g	873.81 g	670 ml	6.70	0.40 ppm
7½ day	1378.40 g	806.36 g	720 ml	6.58	1.90 ppm
10½ day	1312.23 g	767.65 g	705 ml	6.62	5.10 ppm

TABLE 24

CHEMICAL PARAMETERS* IN INTERSTITIAL AND GENERATED PORE WATER FOR SITE 4R

Time	COD (mg/l)	Fe (mg/l)	Mn (mg/l)	Ni	Pb	Cu	Cr	Zn	Cd	Inorganic Hg	Total Hg	PCB
Initial	138	12	2.3	<2	7	23	6	13	<0.5	<0.05	0.2	11
12 hr.	797	10	1.1	12	150	96	7	91	1.7	1.5	1.9	28
1.5 day	667	7	1.0	14	75	123	21	56	2.4	2.7	3.3	14
3.5 day	>1000	11	1.4	19	179	166	17	91	2.0	1.2	1.4	54
7.5 day	423	5	1.0	20	37	94	5	80	1.8	0.2	0.2	17
10.5 day	318	5	1.3	20	35	98	10	94	1.2	---	---	10
15.5 day	130	3	2.1	23	8	56	4	66	1.9	0.3	0.9	14
21.5 day	117	2	2.2	25	2	126	7	130	0.8	0.2	0.2	6

*Values are in µg/l unless designated otherwise.

TABLE 25

CHEMICAL PARAMETERS* IN INTERSTITIAL AND GENERATED PORE WATER FOR SITE 6R

Time	COD (mg/l)	Fe (mg/l)	Mn (mg/l)	Ni	Pb	Co	Cu	Cr	Zn	Cd	Inorganic Hg	Total Hg
Initial	435	28.0	3.58	3.0	6.8	<0.5	17	6.7	56	<0.05	0.22	0.35
12 hr.	1508	12.7	1.94	33.7	119	0.9	173	40.7	156	3.0	1.42	1.82
1.5 day	1316	12.9	1.75	18.9	137	0.8	160	46.0	62	1.8	1.06	1.13
3.5 day	608	2.3	0.87	17.5	2.0	0.6	83	7.1	96	0.95	1.59	1.43
7.5 day	232	5.8	1.82	30.0	24	<0.5	37	8.5	88	1.05	7.5	8.89
10.5 day	163	5.3	1.66	25.0	24	0.6	71	11.0	64	1.2	0.27	0.32

*Concentrations are in μg/l unless designated otherwise.

TABLE 26

CHEMICAL RELEASES FROM SITE 4R SEDIMENT

	COD		Fe		Mn		Ni		Pb		Cu	
Time	mg released	mg*/kg	mg released	mg/kg	mg released	mg/kg	µg released	µg/kg	µg released	µg/kg	µg released	µg/kg
Initial	113	97	9.8	8.4	1.9	1.6	<2	<1.4	5.7	4.9	19	16
12 hr.	413	334	5.2	4.2	0.6	0.5	6.2	5.1	82	67	50	41
1.5 day	300	289	3.1	3.0	0.4	0.4	6.3	6.1	34	32	55	53
3.5 day	>390	>438	4.3	4.8	0.5	0.6	7.4	8.3	70	78	65	73
7.5 day	193	279	2.3	3.3	0.5	0.7	9.1	13.2	17	25	43	62
10.5 day	137	230	2.2	3.6	0.6	0.9	8.6	14.4	15	26	42	71
15.5 day	65	119	1.5	2.7	1.0	1.9	11.5	21.1	4	7	28	51
21.5 day	52	107	0.9	1.8	1.0	2.0	11.0	22.8	1	2	55	115
	Total	1900	Total	32	Total	8.6	Total	91	Total	242	Total	480

	Cr		Zn		Cd		Inorganic Hg		Total Hg		PCB	
Time	µg released	µg/kg	µg released	µg/kg	µg released	µg/kg	µg released	µg/kg	µg released	µg/kg	µg released	µg/kg
Initial	5	4	11	9	<0.4	<0.4	<0.04	<0.04	0.14	0.12	9	8
12 hr.	4	3	47	39	0.9	0.7	0.78	0.64	0.98	0.81	14	12
1.5 day	9	9	25	24	1.1	1.0	1.22	1.17	1.49	1.43	7	6
3.5 day	7	7	35	40	0.8	0.9	0.47	0.53	0.55	0.61	21	24
7.5 day	2	3	36	53	0.8	1.2	0.09	0.13	0.09	0.13	8	11
10.5 day	4	7	40	68	0.5	0.9	---	---	---	---	4	7
15.5 day	2	4	33	61	0.9	1.7	0.16	0.28	0.45	0.83	7	13
21.5 day	3	6	57	61	0.3	0.7	0.08	0.16	0.08	0.16	3	5
	Total	43	Total	350	Total	7.1	Total	3	Total	4	Total	86

*Release per kg of dry sediment.

TABLE 27

CHEMICAL RELEASES FROM SITE 6R SEDIMENT

	COD		Fe		Mn		Ni		Pb		Co	
Time	mg released	mg/kg*	mg released	mg/kg	mg released	mg/kg	µg released	µg/kg	µg released	µg/kg	µg released	µg/kg
Initial	333	216	21.4	13.9	2.74	1.78	2.3	1.5	5.2	3.4	<0.38	<0.25
12 hr.	889	776	7.5	6.5	1.15	1.00	19.9	17.3	70.2	61	0.53	0.5
1.5 day	743	661	7.3	6.5	0.99	0.88	10.7	9.5	77.1	69	0.45	0.4
3.5 day	407	466	1.5	1.8	0.58	0.67	11.7	13.4	1.3	1.5	0.40	0.5
7.5 day	167	207	4.2	5.2	1.31	1.63	21.6	26.8	17.2	21.4	<0.36	<0.5
10.5 day	115	150	3.8	4.9	1.17	1.52	17.6	23.0	16.8	21.9	0.42	0.5
	Total	2480	Total	38.8	Total	7.48	Total	91.5	Total	178	Total	2

	Cu		Cr		Zn		Cd		Inorganic Hg		Total Hg	
Time	µg released	µg/kg	µg released	µg/kg	µg released	µg/kg	µg released	µg/kg	µg released	µg/kg	µg released	µg/kg
Initial	13	8.2	5.1	3.3	43	28	<0.04	<0.03	0.17	0.11	0.27	0.18
12 hr.	102	89	24.0	21	92	61	1.8	1.50	0.84	0.73	1.07	0.93
1.5 day	90	80	26.0	23	35	31	1.0	0.90	0.60	0.53	0.64	0.57
3.5 day	55	63	4.8	5.5	64	74	0.64	0.73	1.07	1.22	0.96	1.10
7.5 day	27	33	6.1	7.6	63	79	0.76	0.94	5.37	6.7	6.4	7.94
10.5 day	50	65	7.8	10.1	45	59	0.85	1.10	0.19	0.24	0.23	0.30
	Total	338	Total	70.5	Total	332	Total	5.2	Total	9.5	Total	11.0

*Release per kg of dry sediment

rough measure of the amount of metals, COD material and PCBs which could be readily flushed from the sediments upon disposal in a clean water system. Since the sediments and added Lake Superior water were intimately mixed by blending, the amounts of chemicals in the water system are probably upper limits to the amount that would be found in pore water moving through the sediments. The results for sites 6 and 4R showed that many of the metal concentrations remained high in the water extractant even after seven extractions. Consequently, significantly greater amounts of these metals (particularly manganese, nickel, copper, zinc and mercury) could have been removed from the sediment with more extractions.

Some additional work was performed using polycarbonate centrifuge bottles to hold the sediment in place of stainless steel. The change in centrifuge bottle material had little effect on the COD, arsenic, cadmium, chromium, cobalt, copper, lead, mangenese, nickel and zinc concentrations in the water extractant. Some enrichment of iron in the water was indicated using stainless steel but this enrichment was not large compared to the magnitude of the iron concentrations.

The times chosen for allowing the Lake Superior water to remain in contact with the sediments were arbitrarily picked. Some limited measurements indicate that the concentrations of the chemical parameters in the pore waters were attained within a few hours of mixing Lake Superior water with the sediment. One experiment on the effect of blending showed that this process greatly increased the concentrations of chemical parameters (COD and metals).

Using the total release values per kg of sediment for the parameters given in Tables 26 and 27 and the total concentrations in the sediments from Tables 2, 9 and 11, the percentage of each parameter extracted by Lake Superior water can be computed. These values are summarized in Table 28. The results show that one per cent or less of the most of the

TABLE 28

TOTAL AND WATER EXTRACTED CHEMICAL PARAMETERS IN SEDIMENTS*

Site 4R	COD	Fe	Mn	Ni	Pb	Cu	Zn	Total Hg	PCB
Total in Sediment	137,000	46,900	685	57	94	40	315	0.77	2
Water Extracted	1,900	32	8.6	0.091	0.242	0.480	0.350	0.004	0.086
% Extracted	1.4	0.07	1.3	0.16	0.26	1.2	0.11	0.5	4.3
Site 6R									
Total in Sediment	168,000	42,400	545	125	84	19	218	0.23	---
Water Extracted	2,480	38.8	7.5	0.092	0.178	0.338	0.332	0.011	---
% Extracted	1.5	0.09	1.4	0.07	0.21	1.8	0.15	5.8	---

*All values of the parameters are in mg/kg.

chemical parameters in the sediments were removed by the water extractions. Only the PCB value for sediment 4R (4.3% extracted) and the mercury value for sediment 6R (5.8% extracted) exceeded the two per cent value. These results indicate that these substances are not readily available to water flushing the sediments unless perhaps substantial amounts of particulate matter is suspended in the water.

SECTION 8

BIOASSAY TESTS

GENERAL BIOASSAY PROCEDURES

Light and Temperature Control

All bioassays were conducted in an environmentally controlled area of the University of Wisconsin-Superior wet laboratory. The area was lighted by an equal number of GroLux Wide Spectrum and Duro-Test Vitilight fluorescent tubes on a 16 hour photoperiod. Light intensity over the bioassay test area averaged 85 ftc. Temperature during the bioassays was maintained between 18-20°C by the facilitie's air conditioning system. Tests were conducted in water baths with temperatures of 17.5-18.5°C. Bath temperature was maintained by control units designed by Mr. Walter Dawson, US EPA Environmental Research Laboratory, Duluth.

Daphnia Culturing Techniques

The parent Daphnia magna stock was obtained from a clone maintained by the US EPA Environmental Research Laboratory-Duluth. Daphnia were cultured in Lake Superior water in 4 liter glass containers held at 18°C in the water baths following published procedures (Biesinger and Christensen, 1972). Approximately 5-10 daphnids were transferred using a 10 mm bore glass tube to the initial 10 culture jars in which 3 liters of Lake Superior water had been allowed to equilibrate to laboratory temperature and pressure for 12-24 hr. Cultures were fed a concentrated mixture of Cerophyll and enriched trout fry granules (Glencoe Mills) blended together with Lake Superior water, at the rate of 1 ml/liter, twice weekly (Biesinger and Christensem, 1972). Lake Superior water used in both culturing and testing was obtained from the EPA Environmental Research Laboratory-Duluth. Daphnia from mature cultures were transferred to additional culture jars to increase the number of animals for testing. Existing cultures were thinned and transferred to new containers containing clean Lake Superior water each week to prevent overcrowding, male production, crashes and bacteria or algae buildup. On the day prior to starting each bioassay, all young daphnids were removed from the stock cultures. This removal insured that young produced during the 24 hr. period proceeding the test could be identified and removed from the stock cultures during the day the tests were initiated. The 20-30 stock cultures generally produced adequate numbers of young daphnids to fill the requirements for each test. However, in a few instances, some two day old animals were mixed in with young of the day. Young daphnids were randomly selected for distribution among the test chambers.

Acclimation and Handling of Test Organisms

Mayfly nymphs, bluegills and *Pontoporeia affinis* collected in the field (SECTION 6) were acclimated to laboratory conditions from 4 days to six weeks prior to being used in bioassays. All animals were acclimated in flow-through aquaria systems. The holding systems were supplied with temperature controlled dechlorinated city of Superior water. The city's water supply is derived from a series of shallow horizontal wells extending out under the bed of Lake Superior from Minnesota Point, and is generally similar in chemical makeup to Lake Superior water (Appendix C).

Mayfly nymphs were acclimated in a 181 x 35 x 40 cm stainless steel chamber located in the area where the bioassays were conducted. The bottom of the chamber was covered with approximately 12 cm of sediment extracted from the area where the nymphs were collected, prior to placing the nymphs and water from the same area, into the chamber. Flow of laboratory water into the chamber was adjusted so that water temperatures were not altered by more than 1-2°C daily until a temperature of 18°C was attained. Once the acclimation temperature of 18°C was reached, temperature control units were used to control temperature and flow in the system. The control units responded to any increase in temperature by opening a solenoid valve resulting in flow of cooler water until the temperature declined to 18°C and the solenoid closed. Oxygen in the chamber was maintained near saturation by air stones placed at both ends.

Mayfly nymphs were removed from the acclimation chamber on the day prior to initiation of each bioassay. The removal process involved gently washing the nymphs from the sediments by placing sediment from the acclimation chamber on screens which were gently agitated in 18°C laboratory water. The agitation served to wash away and float the nymphs free of the sediment. The nymphs were floated off the screens and transferred into a 120 x 18 x 20 cm glass aquaria until the bioassays were started. Temperature, water flow and oxygen in the glass chamber were maintained in the same manner as described for the larger stainless steel acclimation chamber. During the process of removal, nymphs in their last instar, which was distinguished by the presence of large dark wing pads, were discarded. This eliminated the possibility of animal loss through hatching during the bioassays.

Bluegills were acclimated using a system identical to that used for acclimation of mayfly nymphs. However, the chamber was located in a laboratory area where human activity, lighting and room temperature were subject to less control. During the acclimation period, lighting was provided through the laboratory window. Bluegills were treated with a mixture of Malachite green and formalin to control disease subsequent to arrival in the laboratory. A concentrated solution of formalin and Malachite green was added at the rate of 1 ml/liter following the recommendations of Schachte (1974). Fish were maintained in the holding system for a minimum of one week following treatment, prior to being used in the bioassays. During the acclimation period, bluegills were fed commercial trout pellets on alternate days.

Bluegills were removed from the holding system three days prior to initiating the bioassay and placed in the laboratory area test chambers used during the tests to promote acclimation to the test system.

Pontoporeia collected from Lake Superior (SECTION 6) were acclimated in a fiberglass chamber measuring 150 x 27 x 35 cm. *Pontoporeia* were added to the holding chamber on top of a 0.5 cm layer of Lake Superior sediment. Temperature was increased from 12°C to 18°C over a two day period and was maintained at 18°C in the manner described for *Hexagenia*. Oxygen was maintained near saturation by air stones and flow of laboratory water through the system during the 1-2 week acclimation period. *Pontoporeia* used in the tests were collected from the holding chamber by gently stirring the bottom sediments and passing a fine mesh net through the turbid water.

OVERSEDIMENT BIOASSAY

Methods

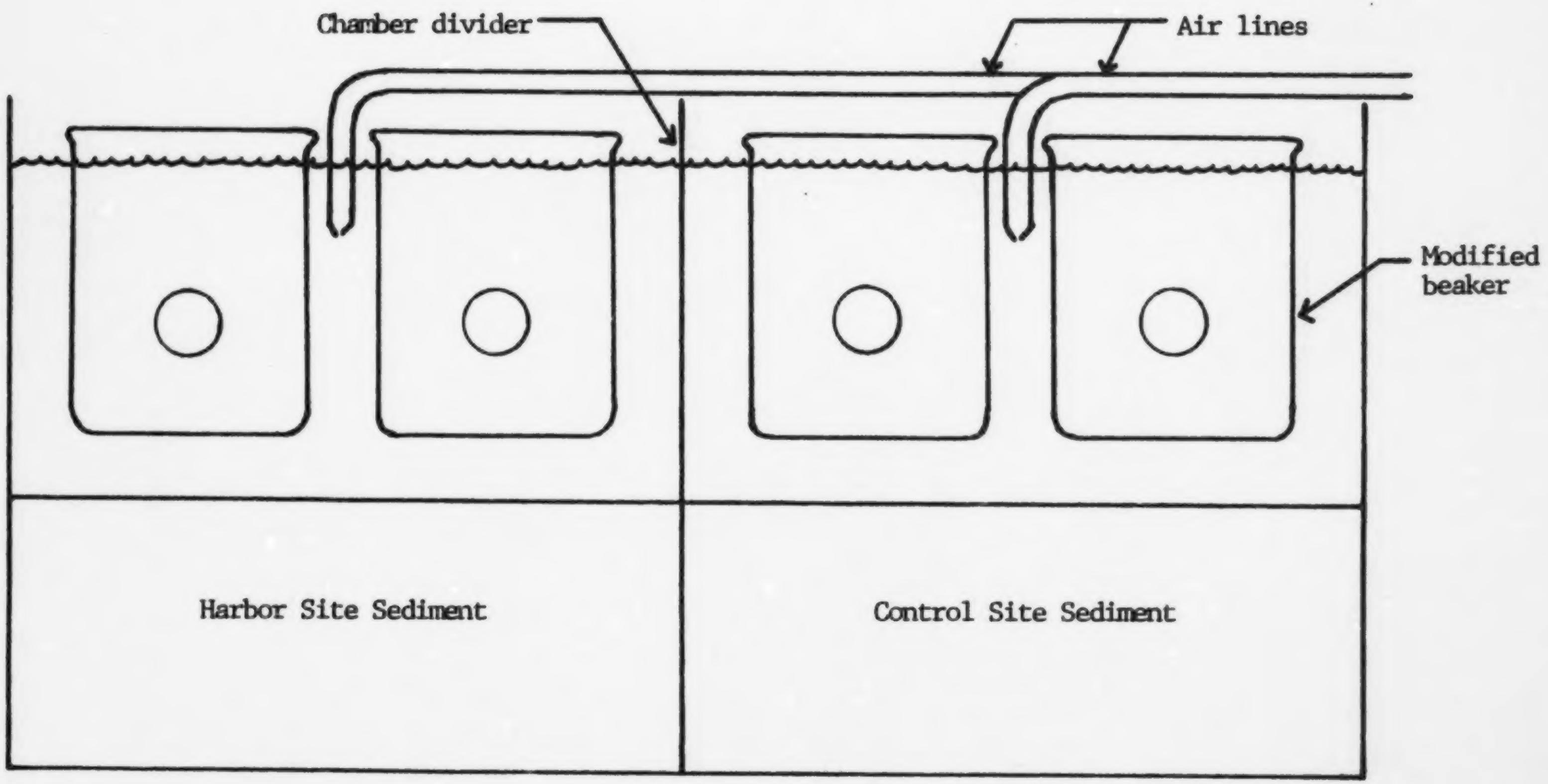
Oversediment bioassays were conducted with sediment from all test sites collected during 1977-1978. *Hexagenia limbata*, *Daphnia magna* and *Pontoporeia affinis* were used in the tests. The basic tests were designed to incorporate the mechanisms for transfer of toxic substances between benthic and plankton communities. In these bioassays, *Hexagenia* served as a sediment toxicity probe, sediment toxicant transport mechanism and a means of measuring availability and bioaccumulation of toxicants. *Daphnia* served as an indicator of toxicity of materials released from the sediments as a result of chemical transfer and activity of *Hexagenia*. *Pontoporeia* bioassays were conducted to measure acute toxicity of liquid phase elutriate water. In these tests, the particulates removed by centrifuging the suspended phase were used to form a sediment base (substrate) for the animals, resulting in the designation as oversediment bioassays.

Hexagenia-*Daphnia* Bioassay Methods--

Most *Hexagenia* and *Daphnia* bioassays were conducted in eight 40 x 20 x 20 cm glass chambers partitioned into two 20 x 20 x 20 cm sections by a removeable divider (Figure 4). One side of each chamber was filled in the field with approximately 8 cm of sediment collected by ponar dredge from the test site. The filled chambers were covered to protect the sediment from light and to control temperature fluctuations during the return trip to the laboratory where an equivalent amount of sediment from the control site was added on the opposite side of the divider. Sediments from the Pokegama control site were normally sampled on the same day or on the previous day with a ponar dredge and held in the laboratory in polyethylene bags at 18°C. In loading the chambers, only sediments which had not been in contact with the dredge were employed. When Lake Superior sediments were used as the control (1978 bioassays), both the control and test site sediments were collected several days to a week prior to use in the tests. The sediments were stored in polyethylene containers at 4°C in the dark until placed in the bioassay tanks. The filled chambers were placed in a water bath at 18°C and covered for approximately 12-15 hours prior to testing.

The first five tests were designed to determine whether *Hexagenia* burrowing behavior could serve as an indicator of sediment quality. During the tests, 4-8 cm of Lake Superior water was added to both sides of the chambers and the divider was removed. *Hexagenia* were added, five over the test sediment and five over the control sediment, one at a time and the point of burrowing was recorded. After the initial ten insects were added the divider was inserted, the overlying water was changed and additional *Hexagenia* were added so that each side should have contained an equal number. Because the insects did not inspect the bottom prior to burrowing, burrowed withing a few seconds of reaching the bottom, and were injured on occasion by placement of the divider, subsequent tests were conducted with the divider in position and placing an equal number of *Hexagenia* on each side of the test chamber. Most tests were conducted with 10 animals per chamber section. However, numbers varied between 5 and 12 according to availability of test

FIGURE: 4
SEDIMENT BIOASSAY CHAMBER


Chamber divider
Air lines
Modified beaker
Harbor Site Sediment
Control Site Sediment

animals in the laboratory. During all tests, individuals which did not actively swim and burrow within three minutes of being placed in the chamber were removed and replaced.

After placing the insects in the chambers, two 250 ml beakers were suspended in the water over the sediments on both sides of each chamber (Figure 4). Each beaker contained two 25 mm holes covered by 50 μ stainless screen attached on the inside with silicone sealant. The modified beakers allowed for water exchange but prohibited escape of young daphnids. Five young of the day *Daphnia* were placed in each beaker at the start of each test.

Oxygen concentration in the test chambers was maintained and controlled so that half the replicates (4) were near saturation (6-9 ppm) and the other half were kept between 1-5 ppm during the 1977 bioassays. Oxygen was maintained near saturation in all chambers during 1978. Oxygen levels were adjusted by controlling air flow through glass pipettes placed in each chamber (Figure 4). Oxygen concentration and temperature were measured four times each day throughout the 16 hour photoperiod with a Yellow Springs Instrument Model 54A system, to identify changes and minimize variation. Oxygen flow was controlled by standard aquarium air valves. Water turbidity in the test chambers was increased by the activity of *Hexagenia* and was measured each day. Turbidity measurements were made with a Nephelometer (Ecologic Instrument Co.) in Formazin Turbidity Units (FTUs). Oxygen, temperature and turbidity means and standard deviations during the tests are presented in Appendix C.

Daphnia survivals were determined after 24, 48, 72 and 96 hours. Test beakers were slowly lifted from the stainless steel wire frame used to suspend them and most of the water was allowed to flow out the screened openings without impinging the *Daphnia*. *Daphnia* in the 3-4 cm deep layer of water remaining in the beakers were counted over a light box. Animals which did not move during counting or respond to being picked up by the glass transfer tube were considered dead.

Dead Hexagenia located at the surface were recorded when turbidity levels permitted observation. Total counts of surviving Hexagenia were made at the end of the 96 hr. tests at the time of sediment removal from each side of the partition. The animals and sediment were separated by the sieving procedure described previously. Living Hexagenia removed from each chamber section were rinsed, wet weighed, wrapped in aluminum foil, appropriately labeled and frozen for subsequent selected organic compound and metal analysis.

At the end of the 96 hour bioassay tests, samples of the overlying water were removed and chemically analyzed for pH, total phenols, H_2S, NH_3, TKN, organic N, COD and a number of metals (Appendix C).

Pontoporeia affinis Bioassay Methods--

Bioassays with Pontoporeia were conducted during 1978 in 250 ml polycarbonate centrifuge bottles. During these tests, 200 ml of suspended particulate phase (SECTION 7) was placed in the bottle and centrifuged for 15 minutes at 10,000 rpm. After centrifuging, the appropriate volumes of the liquid phase were removed and replaced with Lake Superior water to form 5, 50 and 100% concentrations of liquid phase-Lake Superior water mixtures. Sediment located on the sides of the tubes was scraped loose and settled to the bottom to provide a sediment substrate for animals used in the test. Tests were designed to compare survival in the three aqueous phase - substrate systems from each test site with survival in identical systems prepared from the Lake Superior control site sediments and in Lake Superior water (without a sediment base). A total of 10 bioassays were performed with sediments from 7 sites. Each test was performed with 3 to 4 replications. In performing these tests, the test and control site centrifuge bottles were randomly assigned space in the $18^{\circ}C$ water bath and held until temperature equilibrium occurred. Oxygen concentrations were brought to near saturation and maintained by air tubes (glass pipettes). After allowing for appropriate temperature - oxygen adjustments, 6 Pontoporeia were randomly selected from the laboratory stock and pipetted into each chamber.

Water temperature and the number of animals observed on the water surface were recorded once daily during the 96 hr tests. Survival was determined from counts of animals sieved from sediment at the end of each test.

Results

Hexagenia Bioassays--

Survival of Hexagenia in sediments ranged from 97.5 to 33% (Table 29). Survival was lowest in both the harbor test site 1 sediment and the Pokegama Slough control site during the bioassay conducted from June 13-17, 1977 (Table 29). Because of limited number of test animals, the test was conducted with 4 rather than 8 replicates (2 per oxygen level). The limited number of replicates or a reduction in strength of the test animals apparently resulted in abnormally low survival. Because of the low survival of control organisms, data from the test were omitted from all statistical analysis.

Survival of Hexagenia did not fall below 77% in any other bioassay conducted during 1977 with Rainy Lake Hexagenia. Analysis of variance (Steele and Torrie, 1960) was used to determine whether Hexagenia survival was influenced by oxygen concentrations during the 1977 bioassays (Blocks) or the sediment in the chambers (Treatments). The analysis suggested that neither factor had a significant influence on survival. Because oxygen concentration did not influence survival, results for replicates subject to varying oxygen conditions were combined in comparing survival between sediments from Pokegama Slough, Lake Superior and various harbor test sites (Table 30). Mean survival was compared by Student's t statistical parameters. Comparison of means for the 1977 bioassays suggested survival was significantly higher in test site 3 sediment than in sediment from Pokegama Slough. The 1977 tests also suggested that survival in site 4 sediment was lower than in Pokegama

TABLE 29

SURVIVAL OF *HEXAGENIA LIMBATA* IN HARBOR SEDIMENTS AT TWO DISSOLVED OXYGEN LEVELS.

Date	Site	Number of Reps.	Animals per Rep.	SURVIVAL %				Value of F	Level of Sign
				Low O_2		High O_2			
				Control	Test	Control	Test		
June 6 - June 10	2	4	9-19	85.5	92.5	92.2	78.0	2.92	NS
June 13 - June 17	1	2	9	61.0	33.0	62.0	72.0	1.83	NS
June 27 - July 1	6	4	9-11	80.5	85.0	82.5	87.5	0.15	NS
July 11 - July 15	3	4	5-11	85.0	93.0	85.0	92.0	0.83	NS
July 18 - July 22	4	3 or 4	5-10	90.0	77.0	90.0	87.0	0.60	NS
July 25 - July 29	5a	4	10-12	84.0	92.5	85.2	87.5	0.33	NS
Aug. 8 - Aug. 12	LS	4	10	97.5	90.0	92.5	95.0	2.15	NS
Aug. 15 - Aug. 19	6R	4	10	85.0	92.5	85.0	85.0	0.67	NS

TABLE 30

SURVIVAL OF *HEXAGENIA LIMBATA* IN HARBOR SEDIMENTS 1977-1978

Date	Site	Reps./Test	No/Rep.	Survival %		Value of t
				Control*	Test	
1977						
June 6-June 10	2	8	9-19	88.9	85.3	0.8
June 27-July 1	6	8	9-11	81.5	86.2	0.7
July 11-July 15	3	8	5-11	85.1	92.8	2.4**
July 18-July 22	4	6-8	5-10	89.9	81.7	2.0
July 25-July 29	5a	8	10-12	84.5	90.0	1.0
Aug 8-Aug 12	LS	8	10	95.0	92.5	1.0
Aug 15-Aug 19	6R	8	10	85.0	88.8	0.7
1978						
June 12-June 16	4	8	10	87.5	70.0	4.6†
June 19-June 23	3	8	10	71.6	80.7	1.6

* Control represents Pokegama Bay sediments during 1977 and Lake Superior sediments during 1978.

**Values declared significant with $P<0.050$

† Values declared significant with $P<0.010$

Slough sediments (P>0.1, Table 30). To establish the validity of the results, bioassays using sediments from test site 3 and 4 were conducted during 1978. During the 1978 tests, Hexagenia from Ox Creek and Lake Superior sediment (control) were used. The results were similar to those in Lake Superior sediment. Survival in sediments from site 3 appeared to be higher than in Lake Superior sediments.

Observation of Hexagenia indicated that the burrowing occurred shortly after making contact with the sediment and that the insects did not inspect sediments prior to burrowing. However, comparison of the behavior of animals dwelling in sediments from site 4 with other sites (during 1977 tests) show a greater tendency for Hexagenia to return to the surface after burrowing in site 4 sediments. Within the 8 chambers, a total of 5 insects returned to the surface from site 4 sediments whereas, not more than 1 insect returned to the surface of the sediments from the other sites during the observation period.

Daphnia Bioassays--

The survival of Daphnia suspended over sediments varied from 50-100% with the lowest survival occurring during the first bioassay. Analysis of variance suggested that survival of Daphnia was not influenced by oxygen concentration during the 1977 tests. However, survival was significantly lower during the first test which was conducted during June 6-10, 1977 (Table 31).

For this first test, survival was low for both the control (Pokegama Slough) and harbor test site 2 sediment. This low survival in the control suggested that some condition related to technique rather than sediment quality influenced the results. Consequently these results were omitted from further analysis.

Comparison of means for the 1977 bioassays by Duncan's multiple range test (Steele and Torrie, 1960) suggested survival of Daphnia was lower in tests using sediments from sites 1, 4, 6 and 6R than in water over Pokegama Slough sediments (Table 31). However, in no individual test were the means from the

TABLE 31

SURVIVAL OF DAPHNIA MAGNA OVER HARBOR SEDIMENTS DURING 1977

				SURVIVAL %			
				Low O_2		High O_2	
Date	Site	Number of Reps.	Animals per Rep.	Control	Test	Control	Test
June 6 - June 10	2	8	5	62.5	52.5	55.0	50.0
June 13 - June 17	1	8	5	67.5	85.0	85.0	62.5*
June 27 - July 1	6	8	5	100.0	92.5	85.0	67.5*
July 11 - July 15	3	8	5	90.0	90.0	72.0	65.0
July 18 - July 22	4	8	5	98.0	65.0*	90.0	82.0
July 25 - July 29	5a	8	5	80.0	82.5	95.0	77.1
Aug. 8 - Aug. 12	LS	8	5	82.5	85.0	92.5	95.0
Aug. 15 - Aug. 19	6R	8	5	87.5	62.5*	92.5	72.5

*Means identified as significant ($P < 0.05$) by Duncan's New Multiple Range Test.

test site for high or reduced oxygen conditions both significantly different from the control site. For site 1, survival was lowest over the test site sediment at high dissolved oxygen (62.5%), low for Daphnia over Pokegama Slough sediment at low dissolved oxygen (67.5%) and highest (85.0%) over site 1 sediment and the control at low dissolved oxygen (Table 31).

To increase precision, observations at different oxygen concentrations were combined (Table 32). Comparison of means for the 1977-1978 bioassays (Table 32) by analysis of variance showed time of the test (Blocks) did not influence survival ($P>0.1$) but sediment source had a significant effect ($P<0.05$). Data from the August 8-12 bioassay was omitted from the analysis because it compared survival for the two control site sediments (Pokegama Slough and Lake Superior).

Comparison of results for the individual bioassays established that survival was significantly lower over harbor sediments from sites 4, 6R (1977 tests) and 3 (1978 test). Tests with sediments from each of these three site areas were repeated to determine the reliability of the testing procedure. Although mean survival was substantially different for the tested control sites for all repeated tests, differences were not identified as statistically significant for any site twice. Survival results for the 1977 bioassays comparing site 3 and 6 sediments with controls showed no statistically significant differences. However, survival differences were significant for the second tests using sediment from these sites. In the 1977 test, Daphnia survival over site 4 sediment was declared significantly lower than survival over Pokegama Slough sediments. However, mean survival of Daphnia was not significantly lower over site 4 sediment than over Lake Superior sediment in the 1978 test.

Variation in the results from the repeated test could be related to variation in the sediments within the site sampled during different time periods or to the precision of the bioassay procedure. To evaluate pre-

TABLE 32

SURVIVAL OF DAPHNIA MAGNA OVER SEDIMENTS 1977-1978

Date	Site	Reps/Test	Survival % Control*	Survival % Test	Value of t
1977					
June 13-June 17	1	16	76.3	73.8	0.3
June 27-July 1	6	16	92.5	80.0	1.6
July 11-July 15	3	16	81.3	77.5	0.4
July 18-July 22	4	16	92.5	73.8	2.2**
July 25-July 29	5a	16	87.5	81.3	0.7
Aug 8-Aug 12	LS	16	87.5	90.0	0.4
Aug 15-Aug 19	6R	16	90.0	67.5	2.6**
1978					
June 12-June 16	4	16	96.2	91.3	1.8
June 19-June 23	3	16	97.5	81.3	4.1†

* Control represents Pokegama Bay sediments during 1977 and Lake Superior sediments during 1978.

**Values declared significant with $P < 0.05$.

† Values declared significant with $P < 0.01$.

cision of the procedure, comparisons were made between survival of Daphnia magna in the two test beakers suspended over each sediment in the test. Because Daphnia in the two beakers were subject to identical test conditions, it should follow that survival of animals in one beaker should be positively correlated with survival in the other. To test this assumption a correlation analysis was performed employing data from the 1977 bioassays. The calcualted coefficient based on the entire data set suggested some positive relationship exists. However the coefficient was not strong or significant ($r = 0.16$; $P>0.1$). Because it was noted that survival was generally high (100-80%), a second correlation was conducted employing only observations where survival in one beaker of the pair was 60% or lower. This test was conducted on the assumption that a strong relationship would indicate the ability of the bioassays to identify the presence of toxic effects of sediments where they occurred. The coefficient suggested a weak negative relationship ($r = -0.29$ $P>0.1$). The poor correlation between replicates subject to identical test conditions, suggests high variability and low precision of the bioassays in identifying differences in sediment quality.

Chemical Characteristics of Post-Bioassay Water--

The chemical analysis of the water overlying the sediment at the conclusion of the 96 hr oversediment is presented in Appendix C. The general chemical characteristics of the overlying water samples do not vary widely for the various sediments employed in the tests. This result is consistent with the observation that the animal survival values did not vary widely between sites and no one sediment site sample caused significant decreases in survival compared to the control for both Daphnia and Hexagenia tests.

Pontoporeia Bioassays--

Average survival of Pontoporeia affinis varied between 27.8 in 100% elutriate water from test site 4a sediments, to 95.8 in 100% elutriate water from Lake Superior sediments (Table 33). Analysis of variance was performed

TABLE 33

SURVIVAL OF PONTOPOREIA AFFINIS IN ELUTRIATE WATERS - 1978

Harbor Site	No. of Reps	Animals per Rep	SURVIVAL LS	Lake Superior Elutriate 5%	Lake Superior Elutriate 50%	Lake Superior Elutriate 100%	Harbor Elutriate 5%	Harbor Elutriate 50%	Harbor Elutriate 100%
1	4	6	75*	83.3	95.8	83.3	91.7	79.2	87.5
2	3	6	77.8	94.4	50	88.9	66.7	94.4	72.2
3	4	6	87.5	91.7	95.8	95.8	95.8	91.7	95.8
4	4	6	87.5	79.2	91.7	95.8	83.3	91.7	87.5
4a	3	6	77.8	94.4	50	88.9	94.4	50	27.8*
5R	3	8	79.2	66.7	95.8	87.5	58.3	91.7	83.3
6	3	8	79.2	66.7	95.8	87.5	66.7	95.8	62.5

*Mean is significantly different ($P < 0.05$) by Duncan's New Multiple Range Test.

to compare means from all test sites and the results indicated survival was not influenced significantly by the source of the sediments from which water was extracted or concentration of liquid phase elutriate water ($P>0.1$). In the analysis, average survival in 5, 50 and 100% elutriate water from Lake Superior sediments for all bioassays (which was 82.3, 82.1 and 89.7% respectively) was compared with survival in 5, 50 and 100% elutriate water for each of the harbor sediments tested. The results suggest the test was not effective in measuring any general differences between toxicity of elutriate from harbor or Lake Superior sediments. Duncan's new multiple range test was applied to compare survival of Pontoporeia in Lake Superior water; 5, 50 and 100% elutriate water from lake sediments and; 5, 50 and 100% elutriate water from 7 harbor sites, for individual bioassays. Although averages varied considerably the test showed survival was influenced significantly with 100% elutriate water from site 4a and in Lake Superior water during the first bioassay (Table 33). Failure of the test to identify more significant differences may be due to chemical similarity of water from sediments or low precision of tests in identifying differences. Variation in survival between replicates was high suggesting precision of the test procedure was low.

ELUTRIATE, INTERSTITIAL AND PORE WATER BIOASSAYS

Methods

Daphnia Bioassay--

Daphnia magna bioassays were conducted with interstitial, elutriate and generated pore water in 250 ml beakers containing 200 ml of test solution. Six 96 hr bioassays were conducted during June and July 1977. The 1977 tests compared survival of Daphnia magna in 5 and 50% interstitial water from the test sites, survival in 5 and 50% interstitial water from the Pokegama Slough control site and in Lake Superior water. These tests included 5 replications arranged in a latin-square design (Steel and Torrie, 1960). During the August 8-12 test, 5 and 50% interstitial water and 50% elutriate water from Lake

Superior sediments was substituted for interstitial water from a harbor test site. A second bioassay (August 15-19) also included 50% elutriate water from site 6 (6R) as a test medium in addition to 5 and 50% interstitial water from site 6R, the Pokegama Slough control site and Lake Superior water. The August bioassays included 6 replications arranged in a latin-square design. During October, bioassays were repeated for sites 3 and 4 (designated as 3R and 4R). In the October bioassays test media consisted of 5 and 50% interstitial water and 5 and 50% generated pore water from harbor sediments, 5 and 50% interstitial water from Lake Superior sediments and Lake Superior water. These tests included 5 replications in a completely randomized design.

Tests were repeated for sites 3 and 4 during 1977 because Daphnia magna survival was reduced by the use of oxygen supersaturated water during the first test employing sediment for these sites and site 1. During the earlier test Daphnia came to the surface and died in all the chambers, a condition apparently resulting from supersaturation of the test water medium. A second test with sediment from site 6 (6R) was performed to determine the reliability of the procedure in identifying toxicity of sediments.

Daphnia bioassays were conducted with sediment elutriate water and particulate phase water in mixtures with Lake Superior water during 1978 to determine the reliability of the 1977 test results and to identify possible differences between effects of elutriate, interstitial, generated pore water and particulate phase water on Daphnia survival. Nine bioassays were conducted with elutriate and particulate phase water from six site areas. The tests were conducted with Lake Superior water; 5, 50 and 100% elutriate water from Lake Superior sediment; 5, 50 and 100% elutriate water from harbor site sediments; and 10% particulate phase water from harbor and Lake Superior sediments. Each test condition was replicated 5 times.

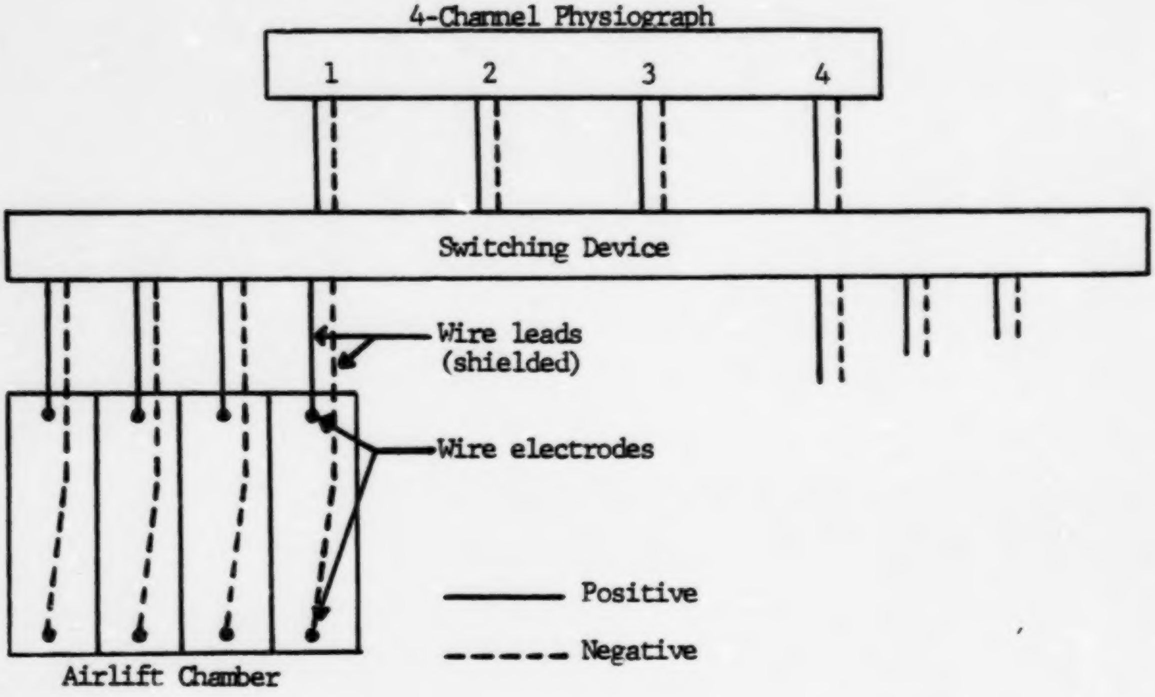
Fish Bioassays--

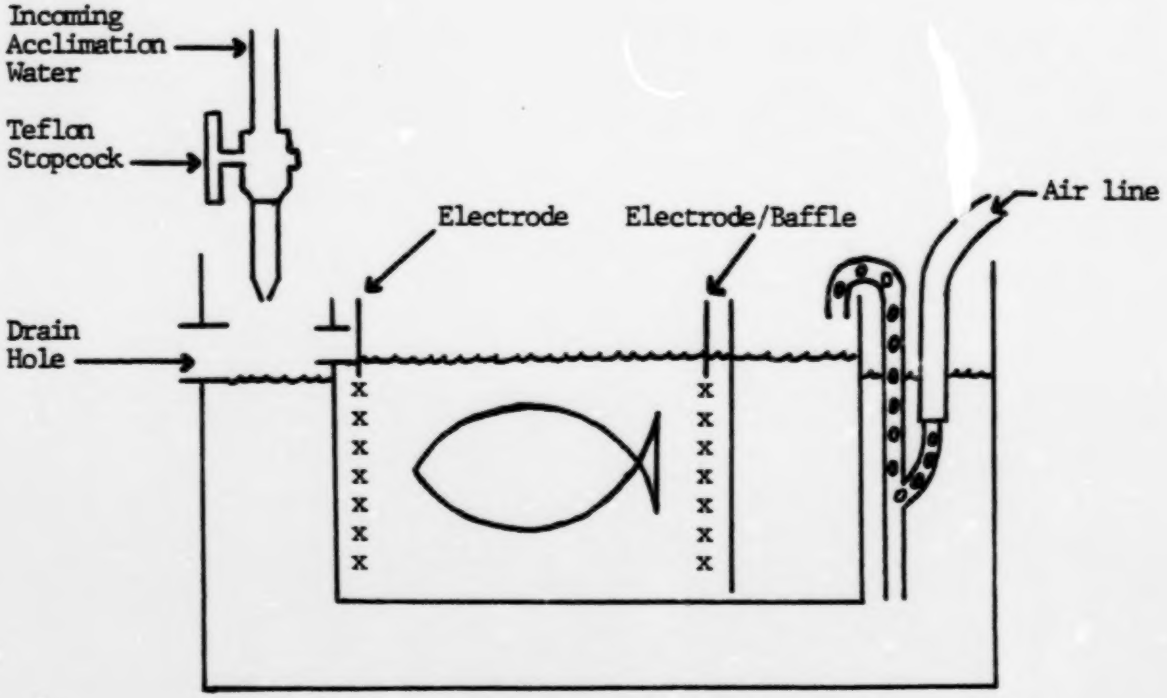
Fish breathing response bioassays (Drummond and Carlson, 1977) were conducted with sediment interstitial water from Pokegama Slough, Lake Superior and harbor test sites 1, 3, 4, 5a and 6R. Sixteen fish of comparable size were used in measuring cough frequency and breathing frequency responses for each bioassay.

The tests were conducted in a system comprised of three major components (Figure 5). Four electrode chambers measuring 11 x 14 x 10 cm were constructed to monitor response from individual fish. Each chamber included four separate 2.5 x 9-12 cm compartments. The linear dimensions of the compartments could be adjusted by a moveable loose fitting partition, from which 4 stainless steel wire electrodes were suspended. Electrodes on the moveable partition and 4 identical ones suspended from the outflow end of the chamber were used to detect action potential (Heath, 1972) resulting from muscular activity associated with breathing. During each test, four bluegill sunfish were placed in each electrode chamber which was suspended in a 3.5 liter (23 x 19 x 14 cm) glass chamber used to hold the test solution. During acclimation and the bioassays, dechlorinated city water, interstitial water or elutriate water was placed in the 3.5 liter chambers and circulated through the grid chambers by an air lift system constructed of glass tubing. Water lifted from the 3.5 liter chamber into the electrode chamber passed by the moveable partition and out through a notch in the top of the opposite end of the chambers. Action potentials picked up by electrodes were amplified and recorded by a Gilson IMP-5H physiograph. The 4 channel physiograph was connected to the electrode chamber electrodes through shielded cable and a rotating switch. The switch rotated at a rate of 1 revolution per hour and switched the physiograph amplifiers to a different electrode chamber every 15 minutes. Switching the changes in electric potential resulting from fish coughs or changes in opercular activity rates were recorded for each fish by the physiograph's 4 channel chart recorder.

During each bioassay, bluegill cough frequency and the percentage of time opercular activity took place,were recorded for fish in dechlorinated city water,

FIGURE: 5
FISH BREATHING RESPONSE BIOASSAY SYSTEM


4-Channel Physiograph
1
2
3
4
Switching Device
Wire leads (shielded)
Wire electrodes
Positive
Negative
Airlift Chamber


Incoming Acclimation Water
Teflon Stopcock
Drain Hole
Electrode
Electrode/Baffle
Air line

Airlift Chamber System

10 and 25% interstitial water from harbor test site sediment and 10% interstitial water from Pokegama Slough sediments. Measurements using dechlorinated water were made after 2½ to 3 days of flow-through acclimation to the water and bioassay system. The measurements for dechlorinated city water established baseline rates under conditions where the air lift system circulated approximately 3 liters of dechlorinated water in the 3.5 liter tank through the electrode chambers. Baseline measurements were recorded over 2 to 3 hr periods 3 times during the day following acclimation. After baseline records were made, portions of the water in the 3.5 liter chambers were removed and replaced with interstitial water to make up 10 and 25% interstitial water mixtures. Tests were conducted with one electrode chamber system (4 fish) in 10% interstitial water from Pokegama Slough, 2 chambers (8 fish) in 10% interstitial water mixture from a harbor test site sediment and 1 chamber (4 fish) in 25% interstitial water from the harbor site. Responses were monitored during several (3 or 4) 2-3 hr periods daily, for three days.

After three days of monitoring response to interstitial water mixtures, the solutions were siphoned from the 3.5 liter chamber and replaced with a steady flow of 18°C dechlorinated Lake Superior water. After 2 hrs the flow of freshwater terminated and breathing response was measured for an additional 2-3 hrs.

Oxygen and temperature of the test solution were measured periodically during periods when response data were not being recorded. At the end of the test fish were weighed and measured (Table 34).

Fish coughs consisted of one or more pronounced opercular movements usually followed by a smaller than normal strength movement and could be identified as a distinct pattern on the physiograph record. Coughs were counted for 10 minutes of each 15 minute recording period for each fish. Experience with bluegills demonstrated opercular activity may be continuous or interrupted by pauses. The percentage of time (during the 10 minute period analyzed) that opercular

TABLE 34

FISH BREATHING RESPONSE BIOASSAY CONDITIONS DATA SUMMARY

Date	Site	Fish Length* cm	Fish Weight* g	Oxygen ppm	Temp °C	Periods Recorded (15 min/Period)		
						Background	Interstitial Water	Elutriate Water
June 10-14	1	8.6-10.4	9.4-17.2	6.6-7.3	17.9-18.7	6	20	---
July 8-15	3	8.5-10.6	7.7-17.4	---	---	12	20	---
July 18-22	4	8.1-10.2	6.9-14.1	6.4-7.1	18.0-18.5	9	20	---
July 25-29	5a	8.6-10.7	7.1-17.6	---	---	9	20	---
August 8-12	LS	8.3-10.5	8.0-16.7	6.6-7.6	18.1-18.2	10	16	---
August 15-21	6R	7.4-9.7	4.2-11.5	---	---	11	21	5

* Measurements made at the conclusion of the tests.

activity took place was determined by counting the number of mm of recorder paper on which opercular movement was indicated and comparing to the total length of the record for the time period.

Results

Daphnia Bioassays--

A latin square analysis was performed on appropriate 1977 data and suggested survival was reduced in interstitial water from sites 5a and 6 (Table 35). Analysis by Duncan's new multiple range test showed survival was lower in 5 and 50% interstitial water extracted from site 6 harbor sediments than in Lake Superior water or interstitial water from the Pokegama Slough control site (Table 35). In bioassays with interstitial water from site 5a, mean survival in 5% interstitial water differed from survival in Lake Superior water and interstitial water from the Pokegama Bay site. During the October bioassays, survival was significantly lower in 5% generated pore water than in 50% generated pore water, Lake Superior water and interstitial water from Lake Superior sediments. Survival in 50% interstitial water extracted from site 3R was also significantly different from survival in Lake Superior water, interstitial water from Lake Superior sediments or survival in 5% interstitial and 50% generated pore water.

Analysis of data from the 1978 bioassays by Duncan's new multiple range test showed mean survival was significantly lower in at least one concentration of elutriate water from harbor sediment sites 1, 3, 4, 5 and 5R than in Lake Superior water or Lake Superior elutriate water (Table 36). Survival was also reduced significantly in particulate phase water from sites 4a and 6. Comparison with the 1977 results suggests that sites 3, 4, 5 and 6 were identified as having a significant negative effect on survival during both years. Survival in tests for site 1 appeared to be lower during 1977 and was significantly lower during 1978 (Tables 35 and 36).

The bioassays failed to show that concentrations of interstitial, elutriate

TABLE 35

SURVIVAL OF *DAPHNIA MAGNA* IN SEDIMENT INTERSTITIAL WATER - 1977

Date	Site	Number of Reps.	Animals per Rep	S U R V I V A L %					Test Elutriate (e) or Generated Pore Water (p)		Value of F	Level of Sign.
				Lake Superior	Control Interstitial		Test Interstitial					
					5%	50%	5%	50%	5%	50%		
June 7 - June 11**	2	5	5	16	16	28	0	32	--	--	--	--
June 14 - June 18	1	3	3	93	93	93	80	80	--	--	0.90	NS
June 28 - July 2	6	5	5	68	68	92	16*	28*	--	--	14.95	0.01
July 12 - July 16**	3	5	5	12	24	52	4	16	--	--	--	--
July 19 - July 23**	4	5	5	28	12	44	0	15	--	--	--	--
July 26 - July 30	5a	5	5	93	100	97	52*	84	--	--	7.97	0.01
Aug. 8 - Aug. 12**	LS	6	5	43	67	60	50	67	--	77e	--	--
Aug. 15 - Aug. 19	6R	6	5	67	83	80	70	73	--	57e	--	--
Oct. 17 - Oct. 21	4R	5	5	96	100	96	92	72*	80p*	100p	--	--
Oct. 24 - Oct. 28	3R	5	5	100	92	100	100	80	56p*	100p	--	--

*Means identified as significant ($P < 0.05$) by Duncan's New Multiple-Range Test.

**Bioassay results influenced by supersaturation of Lake Superior water with oxygen.

TABLE 36

SURVIVAL OF DAPHNIA MAGNA IN ELUTRIATE WATERS - 1978

			SURVIVAL %								
				Lake Superior Elutriate			Harbor Elutriate			Particulates	
Harbor Site	No. of Reps	Animals per Rep	LS	5%	50%	100%	5%	50%	100%	LS 10%	Harbor 10%
1	5	5	96	100	96	96	100	84*	92	96	92
2	5	5	96	100	100	100	88	100	100	96	96
2R	5	5	96	100	100	96	100	100	100	96	92
3	5	5	100	100	96	92	88	68*	64*	92	96
4	4	5	96	96	100	96	92	60*	92	96	95
4a	5	5	96	100	100	96	100	88	96	96	80*
5	5	5	96	100	100	100	96	92	66*	96	84
5R	5	5	96	96	100	100	72*	100	88	92	92
6	5	5	96	96	100	100	96	96	100	92	84*

*Mean is significantly different ($P < 0.05$) by Duncan's New Multiple Range Test.

or pore water from sediments had a significant influence on survival. Means based on survival in 5% interstitial water and 5% pore water appeared to have the same probability of being declared significant as means based on data for 50% concentrations.

Fish Bioassays--

Cough frequency of bluegills in dechlorinated city water, and 10 and 25% interstitial water mixtures from Pokegama Slough, Lake Superior and five harbor test sites (1, 3, 4, 5a and 6R) sediments, were generally similar (Table 37). Higher frequencies which occurred with all mixtures during the first test and with Pokegama Slough 10% interstitial water during the third test, apparently resulted from variation between groups and individual fish used in the test. In addition to variations between fish, cough frequencies varied considerably in time, with the highest frequencies generally occurring shortly after addition of the test mixtures to the system. To control variation between test fish, average cough frequency for each fish in harbor site interstitial water mixtures (12/test) was determined and compared with the background frequency for the specific fish. Analysis of variance showed that variation between fish was significant ($P<0.01$) for several tests (Pokegama Slough, Lake Superior, site 3). The test demonstrated that cough rates were significantly higher than background with interstitial water mixtures from Pokegama Bay, site 1 and site 4 ($P<0.05$). Cough frequencies of fish in interstitial water mixtures from site 5a sediments were significantly lower than background frequencies ($P<0.01$). These differences can be attributed to chemical differences between test mixtures or observation time.

To control time related variation, average cough frequencies were determined for peak activity periods for fish in 10% Pokegama Bay and 10 and 25% harbor or Lake Superior sediment interstitial water. Generally, peak activity occurred during the first 24 hours after introducing the test mixtures. The fish apparently adjusted to the mixtures after 24 hrs resulting in reduced cough frequency thereafter. Peak frequencies (Table 38) were generally higher than averages for the 72 hr tests (Table 37). Comparison of peak cough frequency of bluegills in 10% interstitial water from Pokegama Slough with those for fish in 10 and 25% interstitial water from harbor and Lake Superior sediments showed cough frequency was higher for site 3, 5a and 6R (Table 38).

Bluegill opercular activity occurred almost continuously (91-100%) in dechlorinated water and 10% interstitial water from Pokegama Slough during most bioassays. However, bluegills in 10 and 25% solutions of interstitial water from harbor sites 1, 3, 4, 5a and Lake Superior sediments showed a broken pattern of opercular movement which included periods of inactivity. Comparisons between activity of fish in city water with activity in interstitial water mixtures by analysis of variance showed that the percentage of the time opercular activity took place were significantly lower for all interstitial water mixtures except those from site 6R sediments ($P<0.05$).

COMPARISON OF RESULTS TO OTHER SEDIMENT BIOASSAY STUDIES

Prater and Anderson (1976) conducted sediment bioassays using eight sediments from the Duluth and Superior harbors employing Hexagenia limbata, Arsellus communis, Daphnia magna and Pimephales promelus as biological test organisms. The investigators used an aquarium containing sediment, overlying water and test animals in conjunction with a continuous water recirculation system. The results of 96 hr toxicity tests showed that Daphnia magna generally exhibited the greatest mortality among test organisms in cases where toxic effects were observed. This observation is consistent with the results presented in our study.

Shuba, Tatem and Carroll (1978) conducted bioassay experiments exposing aquatic invertebrates to sediments, standard elutriate water and sediment particulate phases. In these studies, both marine and freshwater organisms were employed as biological probes. The investigators found few cases of statisically significant sediment toxic effects compared to controls even though

TABLE 37

INTERSTITIAL WATER FISH BREATHING RESPONSE

Average cough rates and percentage of the time fish underwent opercular movement is identified for dechlorinated water (Background) and interstitial water from Pokegama Slough (10%) Lake Superior (10+25%) and five harbor sediments (10+25%).

Site	Average Cough Rate (No/Min)					Opercular Activity Time (%)			
	Background		Pokegama	Test Site		Background	Pokegama	Test Site	
	Early	Late	10%	10%	25%	Early	10%	10%	25%
1	2.3	---	2.9	2.7	2.6	99	98	89	80
3	0.8	0.6	0.7	0.7	1.0	91	99	87	53
4	1.1	2.1	3.0	1.0	1.1	100	98	83	68
5a	2.1	0.3	0.8	1.1	1.0	100	69	90	63
LS	0.4	0.8	0.4	0.3	0.8	99	58	56	91
6R	0.2	0.1	0.2	0.2	0.2	97	94	93	92

TABLE 38

PEAK COUGH FREQUENCY OF BLUEGILL SUNFISH IN INTERSTITIAL WATER

Peak cough frequency is estimated as the average frequency during the first 22-26 hrs fish were subjected to interstitial water mixtures.

	Peak Cough frequencies (no/min)		
	Pokegama	Test Site	
	10%	10%	25%
1	2.54	2.62	2.96
3	0.78	1.01*	1.73**
4	1.44	1.09	1.11
5a	1.40	2.10*	2.00
LS	0.28	0.20	0.48
6R	0.13	0.23**	0.15

* Identified as significantly different ($P< 0.05$).

**Identified as significantly different ($P< 0.01$).

many of the test sediments contained high concentrations of contaminants such as metals, PCBs and petroleum hydrocarbons. Their observed mortality results depended upon sediment quality, test organisms used and the length of time the sediment was held in the laboratory. In several cases, repeat studies using the same sediments showing widely different results. This variation in results was also observed in our work as we did not observe significant animal toxicity in repeat determinations using sediments sampled from the same site.

SECTION 9

BIOACCUMULATION POTENTIAL OF SEDIMENT ASSOCIATED CHEMICALS

CHEMICAL ANALYSIS OF HEXAGENIA AND CHIRONOMIDS

The potential for benthic organisms to accumulate organic chemicals and metals in the sediment was investigated by chemical analysis of both bioassay and naturally exposed animals. *Hexagenia limbata*, collected after their use in the 1978 96-hr bioassay tests (Section 8), and chironomids, obtained in 1977 and 1978 from harbor sampling sites, were utilized in the chemical analysis. The animals were analyzed for PCBs, certain pesticides, selected PAH compounds and ten metals.

General Methods

Chironomids collected from the harbor sites (procedure described in Section 6) were dried with tissue paper, weighed, wrapped in aluminum foil and frozen until analysis. Post-bioassay *Hexagenia limbata* were washed in distilled water, dried with tissue paper, weighed, wrapped in aluminum and frozen. Prior to chemical analysis, *Hexagenia* samples were thawed, weighed and dried overnight at 60°C for subsequent dry weight determinations and metal tests.

For organic analysis, one to two gram samples (wet weight) of chironomids and two to three gram samples of *Hexagenia* were ground with 0.5 ml of concentrated HCl using a ceramic mortar and pestle. Anhydrous Na_2SO_4 was added to yield a freely flowing mixture which was transferred to a hexane rinsed cellulose extraction thimble. Each mixture was extracted for 8 hr with 125 ml of a 9:1 mixture (v/v) of hexane-acetone in a Soxhlet apparatus. The extracts were concentrated to 5 ml in a Kuderna-Danish system employing a three ball Snyder reflux column. High molecular weight components were removed by gel permeation chromatography (see Section 7) using methylene chloride as the eluent. For each sample, the lower molecular weight fraction (<600) was concentrated to 5 ml (Kuderna-Danish apparatus) and further concentrated to 2 ml using a gentle stream of N_2 blown over the liquid surface. Aliquots (1.0 ml) of the concentrated extracts were subjected to additional silica gel clean-up and fractionation (see Section 7) to separate PCBs from pesticides and to isolate the PAH compounds in the benzene fraction. The analysis of extracts by gas chromatography and GC-MS (PAH compounds) was carried out by procedures identical to those described for sediment extracts (Section 7).

Chironomids were prepared for metal analysis by placing about 0.05 g subsamples (wet weight) into Parr bombs and adding 2.5 ml of ultrapure HNO_3 to each. The mixtures were digested for one hour at 125°C. After evaporating the mixtures to near dryness, each was diluted to 25 ml with deionized water. The solutions were analyzed by atomic absorption spectrophotometry (Section 7).

Results

The *Hexagenia* and chironomid samples contained some inert material in their digestive tracts since the animals were not placed in clean water for several days after their removal from the sediments (EPA/Corps of Engineers, 1977). This inert material would not be incorporated in tissue and could render bioaccumulation values for tissue either high or low depending upon the concentrations of each chemical constituent associated with the inert material. However the procedure of placing the animals in clean water for several days presents some uncertainty in bioaccumulation values due to possible elimination of certain chemical constituents during this time period.

In order to estimate the amounts of chemicals associated with inert material, residues remaining after HNO_3 digestion of the dried animal subsamples were weighed. These residues represent inert material which varied from 4 to 13% of the total dry weight of animal tissue samples for *Hexagenia* and 4 to 18% for chironomids. The actual weights of sediment in the animal samples prior to digestion in HNO_3 were estimated by adding the weight of that portion of the sediment which dissolved in the digestion process to the residual weight. The determination of the weights of sediment dissolved in the digestion process was based on values determined in the selective extraction procedure for metal analysis (Section 7). It had been determined that the residual fraction of the sediment comprised 80 to 90% of the total dry sediment weight. The measured concentrations of chemical constituents in the animal samples were corrected by subtraction of the amounts associated with the sediments (reported in Section 7) and dividing by the fraction of the total sample weight which represented animal tissue.

Bioaccumulation of PCBs--

Table 39 shows that total PCB concentrations in chironomids from the harbor sites ranged from 6 to 17 μg/g of dry tissue. The dry tissue weight was estimated to be 10% of the wet weight for all samples based on limited measurements. Compared to PCB levels in the dried sediment samples, bioaccumulation factors of 11 to 18 times were found in the animal tissues. PCB values in Hexagenia limbata exposed to harbor sediments for 96 hours were elevated over the value found for the animals prior to their use in the sediment bioassays (Ox Creek value). Bioaccumulation of PCBs was also found for Hexagenia in Lake Superior sediments. The bioaccumulation factors were 4.8 for Hexagenia in the two harbor sediments and 8.2 in the Lake Superior sediment (dry animal tissue/dry sediment).

The amounts of PCBs associated with ingested sediment in the animals were small compared to the total values measured. The corrections for PCBs associated with ingested sediment were 5% or less for Hexagenia and even smaller for chironomids. The effect of correcting for the weights of sediments in the animals was to increase the concentrations of PCBs associated with the animal tissue by 10 to 20%. Consequently, the PCB values measured for the animals containing some ingested sediment were low by 10 to 20% compared to the corrected values given in Table 39.

Bioaccumulation of Pesticides--

Chlorinated pesticides in the sediments were either absent or at very low

TABLE 39. TOTAL PCBs IN SEDIMENT AND BIOLOGICAL SAMPLES (1978)*

	Sediment		Chironomids		*Hexagenia limbata*	
Site	Wet	Dry	Dry Tissue	Magnification Factor	Dry Tissue	Magnification Factor
1	0.16	0.31	6	18	---	---
2	0.32	0.61	10	16	---	---
3	0.64	1.6	17	11	7.7	4.8
4	0.39	1.0	17	17	4.8	4.8
5	0.52	0.85	13	15	---	---
6	0.39	0.77	9	12	---	---
Ox Creek	---	---	---	---	1.7	---
LS[†]	0.15	0.33	---	---	2.7	8.2
LS[‡]	---	---	---	---	4.0	---

* Concentrations in μg/g.

[†] Site 4 *Hexagenia* toxicity bioassay control sediment obtained June 14, 1978.

[‡] Site 3 *Hexagenia* toxicity bioassay control sediment obtained June 22, 1978.

levels, (Section 7) as was the case for the Hexagenia and chironomid samples. The concentrations of DDT, dieldrin, endrin and heptachlor epoxide were below detection limits (<10 ng/g of dry tissue).

Measurable concentrations of only p,p'-DDE were found in the chironomid samples from the harbor sites. These values (in ng/g of dry tissue) were 200 (site 1), 60 (site 2), 140 (site 3), 120 (site 4), 250 (site 4a), and 230 (site 6). Table 11 shows that the p,p'-DDE concentrations in two harbor sediment samples (dry weight basis) were 9.0 and 3.1 ng/g. Comparing these sediment concentrations to the values in the animal tissues shows bioaccumulation of p,p'-DDE in the tissues by factors of about 10 to 40.

Measurable concentrations of p,p'-DDE were found in Hexagenia exposed to harbor and Lake Superior sediments (1977). These values (in ng/g of dry tissue) were 470 (site 1), 230 (site 4), 170 (site 5a), 90 (site 6) and 170 (site LS). Compared to dry weight sediment concentrations (ng/g) of 9 (site 4) and 3.1 (site 6), bioaccumulation factors of 25 and 29 are indicated. For the 1977 Hexagenia bioassay studies, the animals were obtained from Rainy Lake in International Falls, Minnesota. The concentration of p,p'-DDE in a sample of these Hexagenia was about 50 ng/g of dry tissue. The results indicate some bioaccumulation occurred during exposure to harbor and Lake Superior sediments.

Bioaccumulation of PAH Compounds--

Chemical analysis identified PAH compounds in sediments from sites 4 and 6 (Tables 12 and 13). The presence of PAH compounds in chironomids and Hexagenia exposed to sediments from these sites was compared to animals exposed to Lake Superior sediment.

Extracts from chironomids found in site 6R sediment revealed weak MS ion intensity signals for three PAH compounds. The ion signals occurred at molecular weights of 178, 192 and 202. Because of low concentrations, the compounds could not be accurately quantitated but tentative identifications based on the computerized library search and concentration upper limits are given in Table

40. No PAH compounds were found in extracts from chironomids exposed to site 4 sediment.

TABLE 40. PAH COMPOUNDS IN CHIRONOMIDS FOUND IN SITE 6R SEDIMENT.

Retention Time (minutes)	MW	Formula	Compound	Concentration (μg/g)*
11.42	178	$C_{14}H_{10}$	phenanthrene†	<0.47
13.42	192	$C_{15}H_{12}$	methylphenanthrene	<0.83
16.08	202	$C_{16}H_{10}$	fluoranthrene, pyrene	<0.12

* Concentrations expressed on wet weight basis without correction for recoveries through analytical process.

† Identified from retention time only.

The organic extracts from Hexagenia exposed to sediments from sites 4 and 6 showed no presence of PAH compounds. However Hexagenia exposed to Lake Superior sediment had three PAH compounds identified in their tissue extract. An MS ion signal for phenanthrene (anthracene) at a molecular weight of 178 and ion signals for two co-eluting isomers of methylphenanthrene were indicated. The source of these PAH compounds is uncertain since they were not detected in the Lake Superior sediment. No PAH compounds were found in an extract of Hexagenia from Rainy Lake (International Falls, Minnesota). Animals from this location were used in the 1977 Hexagenia bioassays. Table 41 summarizes the PAH results for Hexagenia exposed to Lake Superior sediment.

If these upper limit wet weight concentrations for chironomids are converted to dry weight concentrations based on the estimate that 90% of the total weight of the animals is due to water, upper limit dry weight concentrations of 4.7, 8.3 and 1.2 μg/g are obtained for phenanthrene, methylphenanthrene, and fluoranthrene (or pyrene), respectively. Comparison of these concentrations with the corresponding dry weight sediment concentrations of 0.72, 0.27, and 1.26 (0.85)

(Table 13) yield upper limit accumulation factors of 6.5, 30.7, and 1.0 (1.4) for phenanthrene, methylphenanthrene, and fluoranthrene (pyrene), respectively. In comparison, it has been found that fluoranthene accumulated in oysters (Crassostrea virginica) 694 and 10,000 times after 2 and 8 days of exposure, respectively (Lee *et al*., 1978).

TABLE 41. PAH COMPOUNDS IN HEXAGENIA EXPOSED TO LAKE SUPERIOR SEDIMENT.

Retention Time (minutes)	MW	Formula	Compound*	Concentration (μg/g)[†]
11.34	178	$C_{14}H_{10}$	phenanthrene	<1.38
13.34	192	$C_{15}H_{12}$	methylphenanthrene	<0.27
13.52	192	$C_{15}H_{12}$	methylphenenthrene	---

* Due to weak ion intensity signals, computerized library search was not used but tentative identifications were made from molecular weights and retention time.

[†] Concentrations expressed on wet weight basis without correction for recoveries through analytical process.

Bioaccumulation of Metals--

The concentrations of metals in chironomids collected from sediment at the various harbor sites are shown in Table 42. Based on the residues remaining undissolved after treatment of the animal samples with concentrated HNO_3 in Parr bombs, it is estimated that large percentages of some of these metals were associated with inert material such as the residual phase of sediment particles. More than 25% of the measured concentrations of arsenic, lead, manganese and nickel was estimated to be contained in inert material for at least three different animal samples. In particular for lead, most of this metal was associated with inert material for at lease 5 of the site samples. Consequently, much of the total amount of these metals, as measured in the chironomids, would not be available for bioaccumulation in predators.

TABLE 42. METALS IN CHIRONOMIDS FROM DULUTH-SUPERIOR HARBOR SEDIMENTS.*

Site[†]	As	Cd	Cr	Cu	Fe	Pb	Mn	Ni	Zn	Hg
1	0.6	3.5	4.2	51	1250	11.0	54	25	110	3.8
2	3.2	1.5	11.8	54	11800	3.2	233	40	200	0.9
2R	3.7	4.2	7.2	106	6250	5.6	119	36	58	0.4
3	1.0	1.2	6.5	38	3830	3.2	105	9	500	1.8
4	1.4	0.7	8.9	53	10580	6.3	159	29	152	0.7
5a	1.2	2.2	7.0	35	7120	5.0	146	13	46	4.2
6	2.9	2.0	5.6	35	4840	4.3	547	10	143	9.0
6R	2.8	0.4	10.9	31	11260	18.5	231	14	121	1.6

* Concentrations are μg of metal per gram of wet tissue.

[†] Chironomids were collected from sites 2, 3, 6 and 6R during the spring of 1977. The chironomids from sites 2R, 4 and 5a were collected during the fall of 1977. The chironomids from site 1 were a combined sample from spring and fall collections.

The concentrations of the metals in the chironomids did not show positive correlations with metal concentrations in sediments from the sites. However the sediments exhibited considerable heterogeneous composition at each site and the chironomids were collected from numerous dredge samples at these sites. This presents some uncertainties as to the sediment metal concentrations which were representative of material in contact with the animals. In addition, the levels of metals in the animals may show seasonal variations as indicated by the values for sites 2 and 2R (a repeat sampling of site 2).

The degree of bioaccumulation of metals by the animals from sediments is difficult to assess. However, it is likely that the observed concentrations of mercury, chromium and cadmium were accumulated in the tissues of the chironomids since the amounts associated with ingested sediment were small.

Related Studies

Our results indicated a bioaccumulation of PCBs and DDE by chironomids and *Hexagenia* compared to levels in the sediments. Zitko (1974) found that fish accumulated Aroclor 1254 in their tissues upon exposure to suspended silica particles containing the PCB mixture. Courtney and Denton (1976) observed that the hard-clam (*Mercenaria mercenaria*) accumulated Aroclor 1254 adsorbed on the surface of alumina particles. In both of the above studies, it was found that the lighter chlorinated PCB isomers in the 1254 mixture were preferentially accumulated in the tissues.

Nathans and Bechtel (1977) found that some of the DDT adsorbed on artificial sediments was available for uptake by deposit feeding annelids. Peddicord and McFarland (1978) conducted chemical uptake tests using blue mussels (*Mytilus edulis*), coast mussles (*Mytilus californianus*), soot-tailed sand shrimp (*Crangon nigromaculata*) and dingeness crabs (*Cancer magister*) exposed to contaminated sediment in marine systems. The investigators found that only *Mytilus californianus* took up DDT or its metabolites in the form of DDE. None of these species accumulated PCBs. Studies on uptake of metals indicated slight accumulation of As, Cu, Fe, Pb and Zn from suspensions of contaminated sediment by *Mytilus edulis* and As, Cd, Cu, Zn, Mn and Ni by *Crangon nigromaculata*.

Neff *et al*. (1978) reported accumulation of Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn by *Rangia cuneata*, *Palaemonetes pugio*, *Palaemonetes kadiakensis*, *Neanthes arenaceodentata* and *Tubifex* sp. from sediments during exposures up to six weeks. Some of these species also showed accumulation of Hg and V from sediments. The investigators found variability in metal uptake according to species, salinity of the water, and possibly season. No correlations held between observed bioaccumulation and bulk metal content of the sediment. The authors have reviewed other studies of accumulation of heavy metals from sediments.

Namminga and Wilhm (1977) investigated heavy metals in water, sediment and chironomids from a creek in Oklahoma. They presented evidence that copper and zinc were accumulated in the chironomids compared to water or sediment. Concentrations of 1.91 and 57 μg/g of Cu and Zn respectively were found in chironomids. These values represent bioconcentration factors compared to sediments of 1.1 for Cu and 3.6 for Zn. In contrast, mean concentrations of chromium and lead were lower in the chironomids than found in the sediments.

SAMPLE SCREENING FOR ORGANIC CHEMICALS USING HPLC

A wide variety of natural and industrial orgainc chemcials may be associated with sediments since the partition coefficients pertaining to their distribution between sediment particles and water are often large. The assessment of the amounts of industrially derived organic chemicals associated with sediments is difficult because of their potentially large number and variety. A comprehensive survey results in high costs and the necessity of using a number of analytical techniques. Chemicals of primary concern are those with high lipid solubility (such as PCBs) since these organic compounds tent to bioaccumulate in aquatic animals. The magnitude of the lipid solubility of an organic chemical is related to its bioconcentration in aquatic organisms (Carlson et al. 1975).

Recent studies have demonstrated that the retention time of an organic chemical on a reverse-phase column, as measured using high pressure liquid chromatography (HPLC), correlates with its n-octanol/water partition coefficient (Veith and Austin, 1976; Veith and Morris, 1978; Veith et al. 1979). The logarithm of the retention time was found to be linearly related to the logarithm of the partition coefficient (P) for a large variety of compounds whose lipid solubility properties varied by six orders of magnitude.

The objective of this phase of our study was to investigate the utilization of reverse phase liquid chromatography in screening extracts from sediments and from aquatic animals exposed to the sediments for organic compounds with high lipid solubility.

Methods and Samples

The liquid chromatography system at the Environmental Research Laboratory, Duluth, Minnesota was used. This system has been described elsewhere (Veith and Morris, 1978; Veith et al., 1979). In summary, it consisted of a Varian 4200 instrument employing two 5000-psi pumps, a high-pressure stopflow injector, a fixed wavelength UV detector (254 nm) equipped with a 8-μl flow cell of 1 cm path length and a Varian Micropak® C-10 analytical reverse phase column (250 mm x 2 mm i.d.). The detector was interfaced with a Hewlett Packard 3354 minicomputer for retention time and peak area determinations.

The column was maintained at 50°C during operation. Chemicals under investigation were contained in a 3:1 mixture (by volume) of acetone and cyclohexane (standards used to calibrate column) or hexane (aquatic animal extracts and sediment extracts). Gradient elution was employed by using a solvent system initially consisting of 30% methanol in water and increasing linearly to 100% methanol at the rate of $2\%\cdot\mathrm{min}^{-1}$. Flow rate was $2.0\ \mathrm{ml}\cdot\mathrm{min}^{-1}$ at 3000 psi.

Chemicals move through the column according to their partitioning characteristics and elute in the order of least hydrophobic to most hydrophobic. Consequently those chemicals with the greatest bioaccumulation characteristics (high lipid solubility) were eluted with the longer retention times. Chemicals with log P values ranging from 0.6 to 6.7 were separated and eluted in about 30 minutes employing this gradient elution procedure.

The elution times of chemicals were correlated to their log P values by injecting a mixture of benzene, bromobenzene, biphenyl, bibenzyl, p,p'-DDE and 2,4,5,2',5'-pentachlorobiphenyl. The components of this mixture had log P values of 2.13, 2.99, 4.09, 4.81, 5.69 and 6.11, respectively. Retention times were computed relative to the retention time of phenol which was used as an internal standard.

Portions (20 μℓ) of the extracts from sediments, Hexagenia limbata and Chironomids (Section 7) were passed through the liquid chromatograph. The general operating conditions of the chromatograph were the same as described above. The retention times, peak areas and log P values of eluting compounds were tabulated by the minicomputer. If a retention time matched that of a compound in the computer memory within ± 5%, the tentative identity of the compound was printed along with its retention time.

Results

It was not possible to quantitate compounds by monitoring the UV absorbance of eluting components in the mixtures. The absorptivities of organic compounds at 254 nm can vary by orders of magnitude. Since the eluting compounds were not positively identified they could not be quantitated. Also any compounds with low absorptivities at 254 nm would not be detected. Consequently the only comparisons made between the samples were the number of strong, medium and weak elution peaks at various log P values.

Table 43 summarizes the number of elution bands observed in various log P ranges for sediment, chironomid and Hexagenia samples. The elution bands are qualitatively classified as to being strong (s), medium (m) or weak (w) in terms of relative absorbance at 254 nm.

Comparison of the elution bands for sediment extracts revealed the largest number occurring at log P values greater than 4. Site 2 shows the fewest elution bands with the other sites exhibiting similar numbers. The strongest absorption band in sediment extracts from four of the sites and the second strongest absorption band in extracts from the other two sites occurred at log P = 5.7 which corresponds to the log P value for DDE and also benz(a)pyrene. However the identity of the compound(s) giving rise to this band was not determined.

The chromatograms of the animal extracts show larger numbers of low log P elution bands than sediments corresponding to relatively more polar compounds in the animals. However fewer elution bands with log P>4 values were observed than found for sediment extracts. The strongest absorption band for all the chironomid extracts occurred at log P = 1.5 which may be a naturally occurring compound in the animals. The second strongest absorption band was found at log P = 4.7 which corresponds to the value for bibenzyl. However many other compounds could cause this band. For the Hexagenia extracts, site 2 showed the least number of elution bands similar to the situation for sediments. The strongest absorption bands occurred at log P = 1.4 (sites 5 and 6), log P = 2 (site 2) and log P = 3 (site LS).

TABLE 43. SAMPLE ELUTION BANDS OBTAINED BY REVERSE PHASE CHROMATOGRAPHY.

		Number of Eluted Bands*											
		0 < log P < 2			2 < log P < 4			4 < log P < 6			log P > 6		
Site	Sample Type	S	M	W	S	M	W	S	M	W	S	M	W
1	Sediment	0	0	4	0	0	1	1	3	5	0	5	2
	Chironomids	1	2	3	1	2	2	2	1	1	0	0	0
2	Sediment	1	0	4	0	1	0	2	2	3	0	0	3
	Hexagenia	2	1	4	1	1	1	0	0	0	0	0	2
3	Sediment	0	0	1	0	0	2	3	6	0	0	3	6
	Chironomids	1	2	5	0	1	5	3	1	0	0	0	3
4	Sediment	0	3	4	0	0	4	2	2	7	2	3	3
5A	Sediment	0	1	3	0	0	2	2	6	0	1	2	4
	Chironomids	2	1	4	1	1	0	5	0	0	0	1	1
	Hexagenia	3	1	9	1	3	4	1	4	3	0	0	3
6R	Sediment	0	2	3	0	1	2	5	4	2	1	2	3
	Chironomids	1	0	3	2	1	2	5	2	1	0	0	0
	Hexagenia	2	8	7	1	4	2	1	0	3	0	3	5
LS	*Hexagenia*	1	2	5	2	3	3	2	4	1	0	0	3

* W, M and S refer to weak, medium and strong respectively. The relative intensity ranges used in these classifications are W (0.1 to 1.0), M (1.0 to 3.5) and S (3.5 to 80).

Discussion

The use of reverse phase liquid chromatography shows potential as a useful method in screening environmental samples for chemicals with high lipid solubility. However the large diversity of organic chemicals which may be associated with the sediment particles presents some difficulty in quantitating their amounts. Eluent fractions from various log P ranges could be collected and combined with gas chromatographic and/or mass spectroscopic techniques for identification and quantitation. This procedure would greatly increase the time and expense required in the screening process. Another possibility is to use a fluorescence detector in series with the UV detector for additional monitoring capabilities such as screening for specific PAH type compounds.

Since many industrial organic compounds contain halogens, a determination of such compounds (particularly with higher log P values) would be valuable. This determination might be accomplished by collecting elution fractions in various log P ranges and subjecting these fractions to halogen specific analysis. For example, Glaze _et al_. (1977) used a microcoulometric technique to measure total halogen content of effluents at nanogram levels. The samples screened in this manner could consist of sediment extracts or extracts from benthic organisms after a certain sediment exposure period.

Another possible method which might be employed to arrive at an index of the organic compounds with high lipid solubility in sediments would involve a combined chromatographic and gravimetric procedure. Extracts from sediments or animals exposed to sediments would be cleaned-up by gel permeation chromatography and then chromatographed on a preparative reverse phase column using the method described in this section but with larger injection amounts. After compounds with log P values less than three or four were eluted, the solvent would be changed to pure methanol to remove the rest of the organic compounds (those with high log P values) from the column. This methanol eluent would be collected in tared crucibles and evaporated. The remaining residue would be weighed as a measure of total bioaccuable material. The residue could be subjected to further analysis aimed at compound identification and quantitation if desired. Use of a 20 g sample containing 10 μg/g of high log P compounds would yield 0.20 mg of residue. Consequently, careful microgravimetric methods would be required.

SECTION 10

RELATIONSHIPS BETWEEN CHEMICAL CHARACTERISTICS OF THE SEDIMENT SYSTEMS AND BIOASSAY TOXICITY

SITE LOCATION AND TOXICITY

The complex and variable nature of the sediments and water systems derived from them (interstitial, elutriate and generated pore water) reduced the likelyhood that chemical measurements adequately reflected the complicated chemical conditions in the bioassay test chambers or that a single chemical constituent was responsible for the observed toxicity. Furthermore, variation in survival rates between replicates subject to identically prepared sediment-water systems indicated precision of the bioassay tests were low. Recognizing these limitations, emphasis was placed on development of a general index of toxicity and chemical quality of harbor sediments in trying to identify relationships between sediment quality and animal survival. Because it is possible that some chemical parameters could serve as an indicator of toxicity, an effort was also made to identify correlations between concentrations of specific chemical constituents in the sediments and survival in the bioassay.

Based on the assumption that industrial development within the harbor represents the primary source of toxic substances in the sediments, location of the sampling sites was considered to be a potentially strong indicator of overall sediment toxicity. Application of this simple indicator presented an alternative method to using sediment chemical characteristics to predict toxic effects since the chemical tests may not measure certain toxic substances or the variable toxicity of identified constituents according to their chemical speciation. A general index of toxic effects was developed for each site from the total number of significantly lower mean survival measurements. The total was divided by the number of conducted site bioassay tests to determine the relative percentage of low survival for each site. These percentages are presented for each site in Table 44, and show survival was generally lower in tests representing bioassay systems derived from sediments from sites 3, 4, 5, and 6 (including 3R, 4a, 5a, 6R), which are located in the industrialized areas of the harbor. The percentages show survival was higher in those areas of the harbor which are undeveloped (Pokegama Slough) those influenced by the lake (site 1 and 2) and in the lake proper.

The results suggest that the battery of bioassay tests provide a good general index of harbor sediment quality. The fact that tests employing Daphnia, constantly showed low survival for at least 3 of the 4 industrialized sites indicates Daphnia is the most sensitive among the test organisms employed. Because bioassays with Pontoporeia were the only tests in which survival in Lake Superior water was low, this suggests that the Pontoporeia bioassay results are not reliable.

GENERAL SITE CHEMISTRY AND TOXICITY

To determine whether sites could be generally characterized as to toxic effects from the chemical analysis, sediments from the test sites were ranked on the basis of the results of chemical analysis performed on the sediments or on interstitial water extracted from the sediments. The 7 to 9 sites were assigned ranks from one (representing the site with the lowest concentration of a chemical parameter) to the number of sites considered (representing the site with the highest concentration of the parameter) on the basis of the following:

1. Sediment concentrations (except residual phase metals) of COD, NH_3, TKN, Total S, Total P, Oil and Grease, Total Hg, Pentachlorophenol, As, Cd, Co, Cu, Fe, Pb, Mn, Ni, Se and Zn.
2. Interstitial water concentrations of COD, H_2S, NH_3, TKN, Total Hg, Total PCBs, As, Cd, Cr, Co, Cu, Fe, Pb, Mn, Ni, Se and Zn (1977 data) or As, Cd, Cr, Co, Cu, Fe, Pb, Mn, Ni, Se and Zn (1978 data).

TABLE 44. BIOASSAYS FOR A SAMPLING LOCATION WITH THE NUMBER OF LOW SURVIVAL ESTIMATES DECLARED SIGNIFICANT.

		Bioassay Test with Number of Significant Results								
		Oversediment*		Interstitial**		Elutriate and Pore Water†				
						Liquid Phase		Particulate		
Sampling Site	Number of Tests	Hexagenia	Daphnia	Daphnia	Lempomis	Daphnia	Pontoporeia	Daphnia	Total	Percent
1	6	---	0	0	0	1	0	0	1	17
2,2R	6	0	---	0	0	0	0	0	0	0
3,3R	10	0	1	0	2	3	0	0	6	60
4,4a,4R	13	1	1	1	0	2	1	1	7	54
5,5a,5R	9	0	0	1	1	2	0	0	4	44
6,6R	10	0	1	2	1	0	0	1	5	50
LS	40	0	0	0	0	0	1	0	1	3
Pokegama	28	1	0	---	0	---	---	---	1	4

* Based on data from Tables 30 and 32.

**Based on data from Tables 35 and 38.

† Based on data from Tables 33 and 36.

The general chemical rankings were arrived at by totaling ranks for each parameter and dividing by the number of parameters considered. Regression analysis showed that ranks assigned on the basis of sediment chemistry were not highly reliable in predicting ranks based on intersititial water analysis (Slope = 0.3174; P>0.1). Variation between ranks assigned on the basis of sediment and interstitial water chemistry may result from differential solubility of each chemical in sediments and because concentrations of many parameters were similar for sediments or interstitial water from the sites, reducing the reliability of the ranking.

Rankings based on sediment chemical analysis indicated sediments from the industrialized areas of the harbor (sites 3,4,5,6,6R) were more chemically perturbed than Lake Superior sediments or those from harbor sites 1 and 2 which are located in less developed areas and are strongly influenced by the lake (Table 45). Ranks based on the 1977 sediment chemistry showed the strongest correlation with relative bioassay low survival percentages (Table 44) for each site (r = 0.80; P>0.1). In calculating the correlation, ranks for sites 6 and 6R were averaged. Correlations between relative bioassay low survival percentage and site rankings based on the 1977 and 1978 interstitial water analysis or the average of ranks based on sediment chemistry and interstitial water analysis were not as strong and were not significant (P>0.1).

The analysis suggests that chemical measurements represent a fair predictor of general toxicity and that chemical analysis of sediment quality may be a better indicator of toxicity than interstitial water chemistry.

SPECIFIC CHEMICALS AND TOXICITY

Daphnia was found to be the most sensitive among the animals employed in the bioassay and extensive chemical analysis was performed on *Daphnia* bioassay systems. For these reasons analysis of relationships between concentrations of specific chemicals and toxicity was based upon the *Daphnia* bioassays. The bioassay system for which the greatest amounts of related chemical data were

TABLE 45. SITE RANKING ACCORDING TO SEDIMENT AND INTERSTITIAL WATER CHEMISTRY.

		RANKING				
	1977			1978		
Site	Sediment	Interstitial Water	Average	Interstitial Water	1977-1978 Average	Percent*
1	3.05	3.25	3.20	3.22	3.2	17
2	2.32	4.76	3.54	3.78	3.7	0
3	5.32	5.76	5.54	5.00	5.3	60
4	6.89	4.82	5.86	3.78	4.8	54
4a	---	---	---	4.86	4.9	
5a	4.21	3.23	3.72	---	3.7	44
6	4.84	5.88	5.36	3.89	4.6	50
LS	4.11	5.44	4.77	3.22	4.0	3

* Percent of low survival estimates declared significant (Table 44).

obtained was the system employing Daphnia magna suspended above sediments within bioassay chambers. Consequently, the results from this test system were used to compute correlation coefficients between survival results and concentrations of specific chemical parameters.

The survival values for Daphnia magna over harbor sediments (1977 tests) were given in Table 31. These survival data for Daphnia at high D.O. and low D.O. conditions in Lake Superior water over harbor sediments were used for testing correlations with sediment and water chemistry. Only the survival data pertaining to experiments in which the control Daphnia survival was greater than 80% were used. Student-t values were computed for testing significant differences between mean Daphnia survival above the sample and above the reference sediment (8 replications for each sediment). Six t values were obtained under both high D.O. and low D.O. conditions. The t values, for each set of D.O. conditions, were correlated to sediment chemistry (Table 2, Tables 4 through 9 and Table 11 (Total PCBs)), to interstitial water chemistry (Table 16), to elutriate water from sediments exposed to oxygen (Table 18). Those chemical parameters which showed significant correlations (r exceeds 0.81) are summarized in Table 46.

The correlations results should be considered in conjunction with the uncertainities in the chemical parameters and the limited number of sediment sites tested. Some chemical parameters are near the limits of detection and thus real variation trends are less certain. Other parameters tend to show relatively high fractional standard deviations upon replicate analysis. Even with these considerations, it appears that manganese values may be a good indicator of potential toxicity toward Daphnia in water above the sediments. Positive correlations were found for Daphnia toxicity and manganese concentrations in a number of the sample types (interstitial water, oxic elutriate water, sediment organic phase and total sediment concentration). Larger amounts of manganese in the interstitial water could also indicate the presence of larger amounts of other metals which were associated with $Mn(OH)_3$. However positive correlations of other interstitial

TABLE 46. CHEMICAL PARAMETER-TOXICITY CORRELATIONS FOR *DAPHNIA MAGNA* IN WATER OVERLYING SEDIMENTS

Sample	Chemical Parameter	Oxygen Conditions in Water*	Correlation Coefficient
Sediment	COD	low	0.83
Sediment	NH_3	high	-0.89
Sediment	Total Sulfide	low	0.85
Sediment (organic phase)	Mn	low	0.88
Sediment (organic phase)	Mn	high	0.82
Sediment (residual phase)	As	high	-0.90
Sediment (residual phase)	Ni	low	0.82
Sediment (total metals)	Co	low	0.85
Sediment (total metals)	Cu	high	0.85
Sediment (total metals)	Fe	low	0.81
Sediment (total metals)	Mn	low	0.88
Sediment (total metals)	Zn	low	0.81
Interstitial Water	Cd	low	-0.96
Interstitial Water	Co	high	0.90
Interstitial Water	Mn	high	0.85
Interstitial Water	Ni	low	-0.94
Interstitial Water	Se	high	-0.84
Interstitial Water	Cl	high	0.86
Interstitial Water	Total PCBs	high	-0.88
Elutriate Water (oxic)†	NH_3	low	0.83
Elutriate Water (oxic)	As	low	0.84
Elutriate Water (oxic)	Cd	low	0.82
Elutriate Water (oxic)	Cr	high	0.86
Elutriate Water (oxic)	Pb	low	0.82
Elutriate Water (oxic)	Mn	low	0.81
Elutriate Water (anoxic)	pH	low	-0.84
Elutriate Water (anoxic)	Total phenols	high	-0.81
Elutriate Water (anoxic)	Arsenic	low	0.87
Elutriate Water (anoxic)	Total PCBs	high	-0.95

*Low and high dissolved oxygen values were 1 to 5 mg/l and 6 to 9 mg/l respectively.

†Oxic refers to sediment exposed to air prior to elutriate water preparation while anoxic elutriate was prepared from sediment not exposed to air.

water metals with Daphnia toxicity were not found except for cobalt. Interestingly, a number of metals show positive correlations according to their total concentrations in the sediment.

Relationships were investigated between the concentrations of individual metals in elutriate waters (1978 tests; Table 21) and the survival of Daphnia magna in these waters (Table 35). The percent survival of the animals in the three elutriate water-Lake Superior water mixtures (5%, 50% and 100% elutirate) were compared to survival in corresponding elutriate water control (prepared using Lake Superior sediment) by computing student t values. The t values for the 5% mixtures for all sites (nine t values) were tested for correlations to concentrations of individual metals in both filtered and unfiltered elutriate water. Similar correlations were tested between metals and Daphnia survival for the 50 and 100% elutriate water mixtures.

Interstitial water from the sediments used in the 1978 tests was also analyzed for certain metals (Table 17). These metal concentrations pertaining to either filtered or unfiltered interstitial water were tested for correlations with the t values in the same manner as described using metal concentrations in elutriate water.

Significant correlations ($r > \pm 0.71$) were found between cadmium concentration in unfiltered elutriate water and t values for Daphnia survival in 5% elutriate water-Lake Superior water mixtures ($r = -0.72$) and between copper concentrations in filtered elutriate water and t values for Daphnia survival in 5% elutirate water-Lake Superior water mixtures ($r = 0.71$). However, no significant correlations were identified for tests with 50 and 100% elutriate water mixtures suggesting the instances of correlation may be fortuitous.

More correlations were found between the t values for Daphnia survival and interstitial water metal concentrations. In the case of iron in interstitial waters, r values of 0.80, 0.63 and 0.49 (5%, 50%, 100% elutriate water respectively) were found for unfiltered interstitial water while r values of 0.62, 0.88 and 0.78 were computed for filtered interstitial water. Since three of these values are significant and all are positive, the concentration of iron in the interstitial water may be an indicator of toxicity toward *Daphnia* in elutriate water-Lake Superior water mixtures. Other significant correlation coefficients were found for arsenic (50% mixture, r=0.78), chromium (50% mixture, r=0.74), nickel (50% and 100% mixtures, r=0.97 and r=0.80 respectively) in unfiltered interstitial water.

REFERENCES

Acheson, M.A.; Harrison, R.M.; Perry, R. and Wellings, R.A. Factors Affecting the Extraction and Analysis of Polynuclear Aromatic Hydrocarbons in Water. Water Research, 10:207-212, 1976.

APHA, Standard Methods for the Examination of Water and Wastewater, 17th ed. APHA-AWWA-WPCF, 1975. 1193 pp.

Anderson, B.G. The Toxicity Threshholds of Various Substances Found in Industrial Wastes as Determined by the Use of Daphnia magna. Sewage Works. J. 16:1156-1165, 1944.

Andrew, R.W.; Biesinger, K.E. and Glass, G.E. Effects of Inorganic Complexing on the Toxicity of Copper to Daphnia magna. Water Res. 2:309-315, 1977.

Arthur, J.W.; Andrew, R.W.; Mattson, V.R.; Olson, D.T.; Glass, G.E.; Halligan, B.J. and Walbridge, C.T. Comparitive Toxicity of Sewage Effluent Disinfection to Freshwater Aquatic Life. EPA-600/3-75-012. U.S. Environmental Protection Agency, Duluth, Minnesota, 1975. 61 pp.

Bellar, T.A. and Lichtenberg, J.J. Some Factors Affecting the Recovery of PCBs from Water and Bottom Samples, Water Quality Parameters. ASTM STP 573, American Society for Testing and Materials, 1975, pp. 206-219.

Bernas, B. A New Method for Decomposition and Comprehensive Analysis of Silicates by Atomic Absorption Spectrometry. Anal. Chem. 40:1682-1685, 1968.

Biesinger, K.E. and Christensen, G.M. Effects of Various Metals on Survival, Growth, Reproduction and Metabolism of Daphnia magna. J. Fish. Res. Bd. Canada. 29:1691-1700, 1972.

Bischoff, J.L.; Greer, R.E. and Luistro, A.O. Composition of Interstitial Waters of Marine Sediments: Temperature of Squeezing Effect. Science. 167:1245-1246, 1970.

Boyd, M.B.; Saucier, R.T.; Keeley, J.W.; Montgomery, R.L.; Brown, R.D.; Mathis, D.B.; and Guice, C.L. Disposal of Dredge Spoil; Problem, Identification and Assessment and Research Program Development. Technical Report H-72-8, U.S. Army Engineer Waterways Experiment Station, Vicksburg, Mississippi, 1972. 121 pp.

Bray, J.T.; Bricker, O.P. and Troop, B.N. Phosphate in Interstitial Waters of Anoxic Sediments: Oxidation Effects During Sampling Procedures. Science. 180:1362-1364, 1973.

Breidenbach, A.W.; Lichtenberg, C.F.; Henke, C.F.; Smith, D.J.; Eichelberger, Jr., J.W. and Stierli, H. The Identification and Measurement of Chlorinated Hydrocarbon Pesticides in Surface Waters. PB-215 953, U.S. Dept. of the Interior, Federal Water Pollution Control Administration, Washington, D.C. 1964. 70 pp.

Carlson, R.M.; Kopperman, H.L.; Caple, R. and Carlson, R.E. Structure Activity Relationships Applied. In: Structure-Activity Correlations in Studies of Toxicity and Bioconcentration with Aquatic Organisms, G.D. Veith and D.E. Koncesewich, ed. International Joint Commission Publication, Windsor, Ont. 1975, pp. 57-72.

Carlson, R.W. and Drummond, R.A. Fish Cough Response-A Method for Evaluating Quality of Treated Complex Effluents. Water Res. 12:1-6, 1978.

Cheam, V.; Mudroch, A.; Sly, P.G. and Lum-Shue-Chen, K. Examination of the Elutriate Test, A Screening Procedure for Dredging Regulatory Criteria. J. Great. Lakes Res. 2:272-282, 1976.

Chen, K.Y.; Gupta, S.K.; Sycip, A.Z.; Lu, J.C.S.; Knezevic, M.; Choi, W.W. Research Study on the Effect of Dispersion, Settling, and Resedimentation on Migration of Chemical Constituents During Open-Water Disposal of Dredged Materials, Contract Report D-76-1, Fed., 1976, U.S. Army Engineer Waterways Experiment Station, Vicksburg, Mississippi, 1976. 221 pp.

Corps of Engineers, Draft Environmental Impact Statement. U.S. Army Corps of Engineers Project, Duluth-Superior Harbor. Department of the Army, St. Paul District, Corps of Engineers, St. Paul, Minnesota, 1974. 194 pp.

Courtney, W.A.M. and Denton, G.R.W. Persistence of PCB's in the Hard Clam and the Effect Upon the Distribution of These Pollutants in the Estuarine Environment, Environ. Pollut. 10:55-64, 1976.

DeVore, P. Fishery Resources of the Superior-Duluth Estuary. Center for Lake Superior Environmental Studies, Contract Report #54. University of Wisconsin, Superior, Wisconsin, 1978. 80 pp.

DiSalvo, L.H.; Guard, H.E.; Hirsch, N.D. and Ng, J. Assessment and Significance of Sediment-Associated Oil and Grease in Aquatic Environments. Technical Report D-77-26. U.S. Army Engineer Waterways Experiment Station, Vicksburg, Mississippi, 1977. 146 pp.

Drummond, R.A. and Carlson, R.W. Procedures for Measuring Cough (Gill Purge) Rates of Fish. U.S. EPA Env. Res. Series. 600/3-77-133, 1977. 47 pp.

Drummond, R.A.; Olson, G.F. and Batterman, A.R. Cough Response and Uptake of Mercury by Brook Trout, Salvelinus fontinalis, Exposed to Mercuric Compounds at Different Hydrogen Ion Concentrations. Trans. Am. Fish. Soc. 103:244-249, 1974.

Duchart, P.; Calvert, S.E. and Price, N.B. Distribution of Trace Metals in the Pore Waters of Shallow Water Marine Sediments. Limnol. Oceanogr. 18:605-610, 1973.

Engler, R.M.; Brannon, J.M.; Rose, J. and Bigham, G. "A Practical Selective Extraction Procedure for Sediment Characterization." U.S. Army Engineer Waterways Experiment Station, Vicksburg, Mississippi, 1974. 15 pp.

Environmental Protection Agency/Corps of Engineers, Technical Committee on Criteria for Dredged and Fill Material, Ecological Evaluation of Proposed Discharge of Dredged Material into Ocean Waters. Environmental Effects Laboratory, U.S. Army Engineer Waters Experiment Station, Vicksburg, Mississippi, 1977.

Erikson, C.H. Respitory Regulation in Ephemura simulans Walker and Hexagenia limbata Serville (Epheneroptera). J. Exp. Biol. 40:455-467, 1963.

Fremling, C.R. Mayfly Distribution Indicates Water Quality in the Upper Mississippi River. Science. 146:1164-1166, 1964.

Fremling, C.R. Methods of Mass-Rearing Mayflys (Ephemeroptera:Emphermeridae). Trans. Amer. Fish. Soc. 96:407-410, 1967.

Fuller, F.D. (ed.) Chemical Laboratory Manual of Bottom Sediments. Compiled by Great Lakes Region Committee on Analytical Methods, Lake Michigan Basin Office (Currently EPA Region V Office), Chicago, Illinois, 1969. 101 pp.

Gannon, J.E. and Beeton, A.M. Procedure for Determining the Effects of Dredge Sediments on Biota: Benthos Viability and Sediment Selectivity Tests. J. Water Poll. Cont. Fed. 43:392-298, 1969.

Glaze, W.H.; Peyton, G.R. and Rawley, R. Total Organic Halogen as Water Quality Parameter: Adsorption/Microcoulometric Method. Env. Sci. Technol. 11:685-690, 1977.

Heath, A.G. Critical Comparison of Methods for Measuring Fish Respiration Movements. Water. Res. 6:1-7, 1972.

Heller, S.R. and Milne, G.W.A., EPA/NIH Mass Spectral Data Base. U.S. Department of Commerce, Washington, D.C., 1978.

Helmke, P.A.; Koons, R.D. and Iskander, I.K. An Assessment of the Environmental Effects of Dredge Material Disposal in Lake Superior. Vol. 5. Trace Element Study, Maine Studies Center, University of Wisconsin-Madison, 1976. 148 pp.

Helmke, P.A.; Koons, R.D.; Schonberg, P.J. and Iskander, I.K. Detection of Trace Element Contamination of Sediments by Multielement Analysis of Clay-Size Fraction. Env. Sci. Technol. 11:984-989, 1977.

Keeley, J.W. and Engler, R.M. Discussion of Regulatory Criteria for Ocean Disposal of Dredged Materials: Elutriate Test Rationale and Implementation Guidelines. Miscellaneous Paper D-74-14. U.S. Army Engineer Waterways Experiment Station. Vicksburg, Mississippi, 1974. 13 pp.

Kuehl, D.W., Leonard, E.N. Isolation of Xenobiotic Chemicals from Tissue Samples by Gel Permeation Chromatography. Anal. Chem. 50:182-185, 1978.

Leckie, J.O. and James, R.O. Control Mechanisms for Trace Metals in Natural Waters. In: Aqueous-Environmental Chemistry of Metals, A.J. Rubin (ed.), Ann Arbor Science Publishers, Inc., Ann Arbor, Michigan. pp. 1-76, 1974.

Lee, G.F.; Piwoni, M.D.; Lopez, J.M.; Mariani, G.M.; Richardson, J.S.; Homer, D.H. and Saleh, F. Research Study for the Development of Dredged Material Disposal Criteria. Contract Report D-75-4, U.S. Army Engineer Waterways Experimental Station, Vicksburg, Mississippi, 1975. 337 pp.

Lee, R.F.; Gardner, W.S.; Anderson, J.W.; Rlaylock, J.W. and Barwell-Clarke, J. Fate of Polycyclic Aromatic Hydrocarbons in Controlled Ecosystem Enclosures. Environ. Sci. Technol. 12:832-838, 1978.

Macek, K.J.; Lindberg, M.A.; Sauter, S.; Buxton, K.S.; Costa, P.A. Toxicity of Four Pesticides to Water Fleas and Fathead Minnows. EPA-600/3-76-099. U.S. Environmental Protection Agency, Duluth, Minnesota, 1976. 57 pp.

Magnuson, J.J.; Forbes, A. and Hall, A. An Assessment of the Environmental Effects of Dredged Material Disposal in Lake Superior. Final Rep. Vol. 3, Biological Studies, UW Mar. Stud. Cent., 1225 W. Dayton St., Madison, WI., 1976. 172 pp.

Manning, D.C. and Ediger, R.D. Pyrolysis Graphite Surface Treatment for HGA-2100 Sample Tubes. Atomic Absorption Newsletter. 15:42-44, 1976.

Marzolf, G.R. Substrate Relations of the Burrowing Amphipod Pontoporeia affinus (Amphipoda) in Lake Michigan. Ecol. 46:479-592, 1965.

Namminga, H. and Wilhm, J. Heavy Metals in Water, Sediments, and Chironomids. J. Water Poll. Cont. Fed. 49:1725-1731, 1977.

Nathans, M.W. and Bechtel, T.J. Availability of Sediment-Adsorbed Selected Pesticides to Benthos with Particular Emphasis on Deposit-Feeding Infauna. Contract Report D-77-34. U.S. Army Engineer Waterways Experiment Station, Vicksburg, Mississippi. 1977, 76 pp.

National Biocentric, Inc., Environmental Impact Assessment for Duluth-Superior Harbor. Contract DACW 37-73-C-0074, U.S. Army Corps of Engineers, St. Paul, Minnesota, 1973. 271 pp.

Neeley, W.G.; Branson, D.R. and Blau, G.E. The Use of Partition Coefficients to Measure the Bioconcentration Potential of Organic Chemicals in Fish. Env. Sci. Technol. 8:1113-1115, 1974.

Neff, J.W.; Foster, R.S. and Slowey, J.F. Availability of Sediment-Adsorbed Heavy Metals to Benthos with Particular Emphasis on Deposit-Feeding Infauna. Contract Report D-78-42. U.S. Army Engineer Waterway Station, Vicksburg, Mississippi. 1978, 286 pp.

Nimmo, D.R.; Wilson, P.D.; Blackman, R.R. and Wilson, A.J. PCB Adsorbed from Sediments by Fiddler Crabs and Pink Shrimp. Nature, 231:50-52, 1971.

Olson, G.F.; Mount, D.I.; Snarski, V.M. and Thorslund, T.W. Mercury Residues in Fathead Minnows Pimephales promelas Rafinesque, Chronically Exposed to Methylmercury in Water. Bull. Env. Cont. and Toxicol. 14:129-134, 1975.

Oseid, D.M. and Smith, Jr., L.L. Factors Influencing Acute Toxicity Estimates of Hydrogen Sulfide to Freshwater Invertebrates. Water Res. 8:739-746, 1974.

Panel on Polycyclic Organic Matter, Particulate Polycyclic Organic Matter, Committee on Biological Effects of Atmospheric Pollutants. National Academy of Sciences, Washington, D.C. 1972. 361 pp.

Peddicord, R.K. and McFarland, V.A. Effects of Suspended Dredge Material on Aquatic Animals. Contract Report D-78-29. U.S. Army Engineer Waterway Station, Vicksburg, Mississippi. 1978, 102 pp.

Pezzetta, J.M. Falling-Drop Technique for Silt-Clay Sediment Analysis. Sea Grant Technical Report WIS-SG-72-215. The University of Wisconsin Sea Grant Program, Madison, Wisconsin, 1972. 42 pp.

Prater, B.L. and Anderson, M.A. A 96 hr Sediment Bioassay of Duluth and Superior Harbor Basins (Minnesota) Using Hexagenia limbata, Arsellus communis, Daphnia magna and Pimephales promelas as Test Organisms. Bull. Envir. Cont. and Toxic. 18:159-169, 1977.

Prater, B.L. and Anderson, M.A. A 96 hr Bioassay of Otter Creek (Ohio). J. Water Poll. Cont. Fed. 49:2099-2106, 1977a.

Presly, B.J.; Brooks, R.R. and Kaplan, I.R. Manganese and Related Elements in the Interstitial Waters of Marine Sediments. Science, 158:906-910, 1967.

Robbins, J.A. and Gustinus, J.A. Squeezer for Efficient Extraction of Pore Water from Small Volumes of Anoxic Sediment. Limol. Oceanogr. 21:905-909, 1976.

Rogers, I.H. and Keith, L.H. Identification of Two Chlorinated Guaiacols in Kraft Bleaching Wastewaters. In: Identification and Analysis of Organic Pollutants in Water, L.H. Keith, ed. Ann Arbor Science Publishers, New York, 1976. pp 625-640.

Royse, C.F., Jr. An Introduction to Sediment Analysis. Arizona State University Press, Tempe, Arizona. 1970. 180 pp.

Schachte, J.J. A Short Term Treatment of Malachite Green and Formalin for the Control of Ichtyoputhirius multifillis on Channel Catfish in Holding Tanks. Progr. Fish. Cult. 36:103-104, 1974.

Shuba, P.J.; Tatem, H.E. and Carroll, J.H. Biological Assessment Methods to Predict the Impact of Open-Water Disposal of Dredged Material. Technical Report D-78-50, U.S. Army Engineer Waterways Experiment Station, Vicksburg, Mississippi, 1978. 165 pp.

Snyder, D. and Reinert, R. Rapid Separation of Polychlorinated Biphenyls from DDT and its Analogs on Silica Gel. Bull. Environ. Cont. and Tox. 6:385-390, 1971.

Steele, R.G.D. and Torrie, J.H. Principles and Procedures of Statistics. McGraw-Hill, New York, 1960. 481 pp.

Strosher, M.T. and Hodgson, G.W. Polycyclic Aromatic Hycrocarbons in Lake Waters and Associated Sediments: Analytical Determination by Gas Chromatography - Mass Spectrometry. In:Water Quality Parameters, ASTM STP 573, American Society for Testing and Materials, 1975. pp 259-270.

Thompson, J.F. (ed.). Analysis of Pesticide Residues in Human and Environmental Samples. U.S. Environmental Protection Agency, Research Triangle Park, North Carolina. 1977.

Troop, B.N.; Bricker, O.P. and Bray, J.T. Oxidation Effect on the Analysis of Iron in the Interstitial Water of Recent Anoxic Sediments. Nature. 249:237-239, 1974.

U.S. EPA. Guidelines for the Pollution Classification of Great Lakes Sediments. U.S. Environmental Protection Agency, Region V, April, 1977. 7 pp.

U.S. EPA, Duluth-Superior, Minnesota and Wisconsin. Report on the Degree of Pollution of Bottom Sediment. Region V. Great Lakes Surveillance Branch, Chicago, Ill., 1976. 17 pp.

U.S. EPA, Duluth-Superior, Minnesota-Wisconsin. Report on the Degree of Pollution of Bottom Sediments, 1975 Harbor Sediment Sampling Program. Region V. Great Lakes Surveillance Branch, Chicago, Ill., 1975. 29 pp.

VanTassell, J. and Moore, J.R. An Assessment of the Environmental Effects of Dredge Material Disposal on Lake Superior. Vol. 2. Sedimentation Studies. Marine Studies Center, University of Wisconsin-Madison, 1976. 143 pp.

Veith, G.D. and Austin, N. Detection and Isolation of Bioaccumuable Chemicals in Complex Effluents. In:Identification and Analysis of Organic Pollutants in Water, L.H. Keith (ed.). Ann Arbor Science Publishers, Inc., Ann Arbor, Michigan. pp. 297-302, 1976.

Veith, G.D.; Austin, N.M. and Morris, R.T. A Rapid Method for Estimating Log P Values for Chemicals. Water Res. 13:43-47, 1979.

Veith, G.D. and Morris, T. A Rapid Method for Estimating Log P for Organic Chemicals. EPA-600/3-78-049. U.S. Environmental Protection Agency. 1978. 16 pp.

Walden, C.C.; Howard, T.E. and Froud, G.C. A Quantitative Assay of the Minimum Concentration of Kraft Mill Effluents Which Affect Fish Respiration. Water Res. 4:61-68, 1970.

Whiting, R.J. Report on the Duluth-Superior Harbor 1977 PCB Sampling Program. U.S. Army Corps of Engineers, St. Paul District. 1977, 10 pp.

Winner, R.W. Toxicity of Copper to Daphnids in Reconstituted and Natural Waters. EPA-600/3-76-051. U.S. Environmental Protection Agency, Duluth, Minnesota, 1976. 68 pp.

Wood, J.M. Biological Cycles for Toxic Elements in the Environments. Science. 183:1049-1052, 1974.

Zitko, V. Uptake of Chlorinated Paraffins and PCB from Suspended Solids and Food by Juvenile Atlantic Salmon. J. Env. Cont. and Tox. 12:406-412, 1974.

APPENDICES

A - Analytical Methods of Analysis 146

B - Analysis of PCB Gas Chromatographic Elution Patterns 154
Identification of PAHs 159

C - Chemical Characteristics of Duluth-Superior Harbor Water (Table C-1), Lake Superior and City of Superior Water (Table C-2), and *Hexagenia* Bioassay Water at High D.O. and Low D.O. (Tables C-3, C-4, and C-5). 174

D - Great Lakes Harbor Sediment Evaluation Criteria (Jensen Criteria). 182

APPENDIX A

ANALYTICAL METHODS OF ANALYSIS

ANALYTICAL METHODS OF ANALYSIS

A. Sediment Analysis

The data for the sediment core analysis is given in Tables 2, 3, 9 and 10. The procedure used for analysis of each parameter is described below.

1. Eh. The Eh values for the sediments were measured by inserting a platinum and a silver-silver chloride electrode (4M KCl) into the sediment core to a depth of 2 cm. The millivolt display on the Corning Model 5 pH Meter was read and corrected to a scale corresponding to use of the standard hydrogen electrode as the test electrode.
2. pH. The pH of the sediments was obtained by insertion of a glass and a silver-silver chloride electrode two centimeters into the sediment and reading the value from a Corning Model 5 pH Meter.
3. Particle Size Analysis. Pretreatment of the sediment was done to remove soluble salts and organic material. The pipet-sedimentation method was used to measure dispersed particle settling rates (Royse, 1970). The sample was washed through a #230 mesh sieve into a cylinder using an aqueous solution of a dispersing agent (sodium hexametaphosphate). Aliquots of the suspension were removed from the cylinder at specified distances below the surface as a function of time. The weights of particles in the aliquots were determined by drying them at 105°C and related to the particle size distribution in the sample by using Wadell's practical sedimentation formula tables (Pezzetta, 1972).
4. Chemical Oxygen Demand. A weighed portion of sample was preserved by adding distilled water and sulfuric acid. The sample was refluxed with potassium dichromate in the presence of mercuric sulfate, silver sulfate and sulfuric acid for 2 hours. Unreacted

dichromate was titrated with standardized ferrous ammonium sulfate. The volume of titrant used for the sample was corrected for dichromate loss utilizing reagent blanks carried through the same digestion and titration procedure (Fuller, 1969).

5. Ammonia Nitrogen. A preweighed sample, which was preserved with sulfuric acid, was washed into a Kjeldahl flask and the pH was adjusted to approximately 6.6. The sample was distilled and the distillate was collected in 0.02 N sulfuric acid (Fuller, 1969). The collected distillate was analyzed by adding sodium hydroxide and determining the amount of ammonia produced with an ammonia gas sensing electrode (APHA, 1975).
6. Kjeldahl Nitrogen. The sample was digested with sulfuric acid, sodium sulfate and mercuric sulfate for 30 minutes following the appearance of sulfur trioxide fumes. The solution was cooled, water and a 50% sodium hydroxide-sodium thiosulfate solution were added making the digestate alkaline. The resulting solution was distilled into a 2% boric acid solution to collect the distillate. The collected distillate was analyzed using the same procedure employed for ammonia analysis (Fuller, 1969).
7. Total Solids. The weighed sediment samples were dried in crucibles overnight at 103 to 105°C and reweighed. The change in weights were used to calculate total solids.
8. Volatile Solids. Volatile solids were determined by placing the oven-dried crucibles from the total solids procedure in a muffle furnace at 600°C for one hour. The change in weight of residue after ashing was used to calculate the volatile solids.
9. Total Phosphorus. A sediment sample was digested with a mixture of sulfuric and nitric acids. After digestion the sample was cooled, water was added and it was made slightly basic with sodium hydroxide.

The resulting orthophosphate was determined colorimetrically using the stannous chloride method (APHA, 1975).

10. Oil and Grease. A preweighed sediment sample was acidified with hydrochloric acid to an approximate pH of 2. The acidified sample was then dried by grinding it with magnesium sulfate. The dried sample was placed in an extraction apparatus and extracted for 4 hours with hexane. The hexane was distilled off and the flask was cooled and dried in a dessicator and weighed. The difference in the final and initial weights of the extraction flask were used to calculate the amount of oil and grease in the original sample (Fuller, 1969).

11. Total Sulfide. A sediment sample, previously preserved with zinc acetate, was placed in a distillation apparatus and flushed with nitrogen. Hydrochloric acid was added to the sample to make it acidic. The sample was distilled and the distillate was collected in 0.2 N zinc acetate. Color development was achieved by adding an amine solution and iron (III) chloride to the distillate. The sulfide concentration was determined by comparing the color developed in the sample distillate to that of standards and a blank that were carried through the same procedure (Fuller, 1969).

12. Total Phenols. Sediment samples to be analyzed for total phenols were preserved by adding copper sulfate and phosphoric acid. The preserved samples were diluted to 500 ml with deionized water and distilled to remove the phenols from any nonvolatile impurities. Ammonium chloride was added to the distillable phenols and the pH was adjusted to a value of 10 with ammonium hydroxide. Aminoantipyrine and potassium ferricyanide were added to develop a yellow color. This solution was extracted with chloroform and the extract was analyzed colorimetrically (APHA, 1975).

13. Total Mercury. A one to two gram sample was digested with a mixture of nitric acid, sulfuric acid, a 6% solution of potassium permanganate and a 5% solution of potassium persulfate. The sample was cooled and diluted to volume with deionized water. A sodium chloride-hydroxylamine sulfate solution (reduces excess permanganate) and aqueous stannous sulfate (reduces ionic mercury to elemental mercury) were added and the sample was analyzed. The concentration of mercury was determined by comparing the absorbance of the sample to that of standards and blanks that were treated in the same manner. The instrument that was used for this procedure was a Perkin-Elmer 306 atomic absorption spectrophotometer fitted with a cold vapor mercury analysis apparatus (Olson et al., 1975).

14. Total Metals. The sediment sample was placed in the teflon cup of a Parr acid digestion bomb. A mixture of aqua regia and hydrofluoric acid was added to the sample and the digestion bomb was sealed and heated to 120°C for 90 minutes. The digested sample was cooled and water and boric acid were added to dissolve any metal fluorides that had formed (Bernas, 1968). The digested samples were analyzed using a Perkin-Elmer 306 atomic absorption spectrophotometer equipped with a deuterium arc background corrector and the Perkin-Elmer HGA 2100 graphite furnace. Samples were analyzed by either flame or flameless techniques depending on the concentration of metal in the sample.

B. Water Analysis

The results for chemical analysis of liquid phase samples were listed in Tables 15 through 26. The methods used in these analyses are summarized below.

1. Total Sulfide. An unfiltered water sample was preserved by the addition of zinc acetate. The preserved sample was placed in a

distillation apparatus, the system was flushed with nitrogen and the sample was acidified with hydrochloric acid. The sample was distilled with the distillate collected in 0.2 N zinc acetate solution. The collected distillate was treated with ferric chloride and amine-sulfuric acid reagent for color development. The concentration of sulfide was determined by comparing the absorbance of the sample to that of standards that were treated in the same manner (APHA, 1975).

2. Ammonia Nitrogen. An ammonia electrode (Orion) was used to analyze the sample. Sodium hydroxide was added to the sample and the amount of ammonia present was calculated by comparing the sample to various standards (APHA, 1975).
3. Total Kjeldahl Nitrogen. An unfiltered water sample was treated with sulfuric acid, potassium sulfate and mercuric sulfate as the digesting reagent. The digested sample was analyzed using the same procedure that was previously described for ammonia (APHA, 1975).
4. Organic Nitrogen. This value was calculated as the mathematical difference between the total Kjeldahl nitrogen and ammonia value for the same sample.
5. Chemical Oxygen Demand. A water sample was refluxed with a mixture of potassium dichromate, sulfuric acid, mercuric sulfate and silver sulfate for 2 hours. The remaining unreacted dichromate was titrated with standard ferrous ammonium sulfate. The chemical oxygen demand of the water was calculated by correcting the amount of titrant used for the sample by the amount used for a blank carried through the same procedure (APHA, 1975).
6. Orthophosphate. An unfiltered sample was reacted with ammonium molybdate and stannous chloride. The absorbance of the resulting blue color was compared to that of standards and blanks treated

in the same manner (APHA, 1975).

7. Suspended Solids. The water sample was filtered through a preweighed 0.45 μ membrane filter. The filter was oven-dried at 105°C and reweighed. The difference in the final and initial weights of the filter were used to calculate suspended solid values.

8. Chloride. A titration of the filtered water sample with mercuric nitrate was performed using diphenylcarbazone as the indicator. The volume of titrant used for the sample was corrected for the amount used in titrating a blank (APHA, 1975).

9. Metal Analysis. The acidified aqueous samples were analyzed employing a Perkin-Elmer 306 atomic absorption spectrophotometer equipped with the Perkin-Elmer HGA 2100 graphite furnace and deuterium arc background correction. The samples were run on either the flame or furnace attachments depending upon the concentration of metal in the sample. Concentrations of metal were calculated by comparing the absorbance of the sample to that standards or by standard addition if interferences were encountered.

10. Inorganic Mercury. A digestion of the sample was performed, using a mixture of nitric acid, sulfuric acid and potassium permanganate as the digesting reagent. The resulting solution was treated with a sodium chloride - hydroxylamine sulfate solution and aqueous stannous sulfate. Liberated mercury was measured on an atomic absorption spectrophotometer equipped with a cold vapor mercury apparatus (Olson et al., 1975).

11. Total Mercury. The procedure is the same as that used for inorganic mercury except that potassium persulfate was also added during the digestion procedure (Olson et al., 1975).

12. Organic Mercury. The organic mercury value was calculated as the difference between the total and inorganic mercury concentrations.

13. Dissolved Oxygen. The dissolved oxygen values were determined using a Yellow Springs Instrument Model 54A Dissolved Oxygen Meter. The instrument's probe was placed in the sample and moved slowly until a steady reading was obtained. The instrument was calibrated by the Winkler titration method (APHA, 1975).

14. Specific Conductance. The measurements were made at 25°C using a Freas type conductivity cell and an Industrial Instruments Model RC16B Conductivity Bridge.

APPENDIX B

1. ANALYSIS OF PCB GAS CHROMATOGRAPHIC ELUTION PATTERNS
2. IDENTIFICATION OF PAHs

1. ANALYSIS OF PCB GAS CHROMATOGRAPHIC ELUTION PATTERNS

General Description

The more commonly used commercially available PCB mixtures are designated as Aroclor 1242, 1248, 1254, 1260 and 1262. A total of 23 different polychlorinated biphenyl compounds (a number of which are common to more than one Aroclor mixture) were considered in analyzing samples for these mixtures. The PCB components present in environmental samples and their relative abundances do not match any of the commercial mixtures exactly. However the nature and concentrations of PCB components in an environmental sample may be considered to be a single Aroclor mixture or a linear combination of two or more mixtures whose individual components have been altered by solubility differences or degradation by microorganisms.

The computation of PCB concentrations in the environmental samples were carried out in two ways. The first method involved calculation of the concentration of each of the possible 23 PCB components commonly found in samples[1] (if present) and summing these values to give a total PCB concentration. The second method consisted of determining the best fit of the gas chromatographic elution patterns of the five Aroclor mixtures to the elution pattern of the sample. The concentration of PCBs in a sample was expressed as specific amounts of one or two Aroclor mixtures which best matched the sample elution pattern.

Specific Procedure

Total PCBs--

1. The retention times of the peaks from the sample chromatograms were measured and compared to the retention time of the 23 different PCB peaks on the same chromatogram. Sample peaks matching the retention times of the PCB peaks were tentatively identified as PCBs.

---

[1] Webb, R.G. and McCall, A.C. Quantitative PCB Standards for Electron Capture Gas Chromatography, J. Chrom. Sci., 11:366-373, 1973.

2. A computer program was used to calculate the concentration of PCBs in each sample. Preliminary input information to the computer consisted of:

A. A PCB component response factor for each of the 23 PCB peaks obtained during an earlier series of injections. A response factor is a quantity representing the mm of elution peak height produced by the weight of the chromatogramed standard or sample.

B. A conversion factor that adjusts the individual PCB response factor to the chromatographic conditions of the particular series of samples being analyzed. This conversion is based on the relative response factors of the individual PCB components compared to the response factor of a known weight of aldrin under the same instrumental operating conditions.

3. The specific data that was entered into the computer for calculating the total concentration of PCBs in a sample is listed below:

A. The injection volume of the standard aldrin solution and the resulting peak height due to elution of the aldrin.

B. The peak heights of those elution peaks tentatively identified as PCBs in a given sample.

C. The volume of each sample injected.

D. The initial weight of the sediment sample or initial volume of the water sample and final volume of the sediment or water extract.

E. The factor by which the extract was diluted.

4. The concentration of each PCB component was computed using the following equation:

$$\text{PCB Conc} = (\text{standard conc.}) \frac{(\mu\text{l standard injected})}{(\mu\text{l sample injected})} \frac{(\text{peak height in sample})}{(\text{peak height in standard})}$$

$$\text{X} \quad \frac{(\text{volume of sample extract})(\text{extract dilution factor})}{(\text{weight of sediment or volume of water})}$$

5. The concentrations of the individual PCB components were summed to obtain the total concentration of PCBs.

Aroclor Mixtures--

The computer program contained a procedure designed to analyze the sample component retention times and relative peak heights by identifying the Aroclor mixture or mixtures most closely resembling the components found in the sample. This procedure is summarized as follows.

1. Seven preselected elution bands from each Aroclor standard were used as references for determining the presence of the commercial mixture in the sample. The presence and amount of each mixture was tested in the order 1262, 1260, 1254, 1248 and 1242.
2. The ratio of the peak height of the first elution band (tested band) to the peak height of the first reference band was computed for the standard. If the first elution band (test band) and first reference band were present in the sample, the ratio was also calculated for the sample. If the ratios for sample and standard matched within ±20%, the concentration of Aroclor 1262 was calculated based on the peak height of the tested band of the sample. The computation of ratios of peak heights in the standard and sample based on the first reference band was repeated for any of the other 21 possible PCB elution bands which were present in the sample. For those sample and standard elution band peak height ratios which agreed with ±20%, concentrations of Aroclor 1262 were computed. All calculated Aroclor 1262 concentration values were averaged and this information was printed along with the number of PCB tested band-reference band peak height ratios in the sample that matched elution band-tested band peak height ratios in the standard.

3. The procedure described in step 2 was repeated using the other six reference bands (if present in the sample) chosen in the Aroclor 1262 mixture. The results gave a maximum of six more averaged concentration values for Aroclor 1262 in the sample. All concentration values of Aroclor 1262 based on the seven reference elution bands were then averaged using a weighted average according to the number of sample and standard peak height ratios which matched within ±20% for each reference band. This average was reported as the overall average Aroclor 1262 concentration in the sample.
4. If a larger number of elution bands (generally six or more) from the sample were used in the calculation of the average Aroclor 1262 concentration based on each of the reference elution bands then Aroclor 1262 was assumed to be present in the sample. The peak height contributions due to the presence of the computed concentration of Aroclor 1262 in the sample were subtracted from the observed sample peak heights. However if only a few elution bands were listed as used in the calculation of the Aroclor 1262 average concentrations, then Aroclor 1262 was assumed to be absent in the sample and this subtraction was not made.
5. Steps 2 through 4 were repeated for each of the other Aroclor mixtures in the order 1260, 1254, 1248 and 1242 using the seven selected reference bands for each mixture.
6. After computation of the concentrations of all the Aroclor mixtures and subtraction of the peak heights due to the presence of the mixtures from the observed sample peak heights, the peak heights remaining for each of the 23 elution bands were listed.

For the samples analyzed in this study, it was found that their PCB concentrations could be expressed in terms of one or two of the Aroclor mixtures.

2. IDENTIFICATION OF PAH's

For several samples, benzene eluates from the silica gel fractionation procedure were chromatographed along with selected PAH standards on temperature-programmed runs for comparisons of retention times between peaks in samples and standards. GC separations were made in a Varian 1700 Series instrument equipped with a flame-ionization detector, and a glass column (6' x 1/8" ID) packed with 3% OV-101 on Gas-Chrom Q. The carrier gas flow rate was 15 ml/min. A starting column oven temperature of 80 C was held for 1 min, with a programmed increase of 4 C/min to a final holding temperature of 225 C. Gas chromatograms of sediment extracts from Lake Superior, site 4, site 6, Pokegama Bay are presented in Figures B-1 to B-4.

Identification of PAH's was performed at the U.S. Environmental Protection Agency's Environmental Research Laboratory-Duluth on a Finnigan GC-MS system. A Finnigan Model Model 9610 GC was connected to a Finnigan Model 4032A quadrapole MS via a glass transfer line. A Finnigan INCOS 2300 data system was used on line to acquire and process mass spectral data.

A glass GC column (6' x 1/8" ID) was packed with 3% OV-1 (methyl silicone) on 60/80 mesh Gas-Chrom Q. The following GC conditions were typically employed: helium carrier gas flow rate, 20 ml/min; injector temperature, 250 C; separator oven temperature, 280 C; transfer line temperature, 280 C; and column oven temperature programmed from 100 C to 225 C at 4 C/min.

The mass spectrometer was scanned 50 to 500 at 2.05/decade in electron impact mode (70 ev). The instrument was tuned to provide a 442/198 amu ratio of 0.7-0.9 for the spectrum of Ultramark 443 (decafluorotriphenylphosphine, PCR Research Chemicals, Inc.). Calibration was accomplished with FC43 (perfluorinated tributyl amine).

As an example, Figure B-5 presents a reconstructed ion and selected mass chromatograms for site 4 sediment extract. Ion intensity, or the number of ion strikes on the mass spectrometer detector for a compound at a given mass, is indicated by the top number of each grouping along the right margin of the figure. Ion intensity is related to quantity of compound. The mass chromatogram peaks for the upper lines of the figure are normalized to the compound producing the greatest ion intensity. In Figure B-5, for example, they are all normalized to the ion intensity value of 3064 at MW 202. In the left figure margin there are pairs of numbers associated with the individual mass chromatograms. The lower number of each pair refers to the MW of the compound producing the mass chromatogram, and the upper number refers to percentage ion intensity at a given MW compared to the highest ion intensity of the mass chromatogram displayed.

Mass spectra of unknown compounds from sample extracts were compared to mass spectra of standard compounds in the EPA/NIH Mass Spectral Data Base (Heller and Milne, 1978) by a computerized library search. Each search identified three compounds whose mass spectra best matched mass spectra of unknown compounds. This listing combined with retention time data of several PAH standards provided for a positive identification of several PAH's. Examples of library search results are presented in Figures 6 through 13 for unknown compounds at particular masses in the sediment extract from site 4.

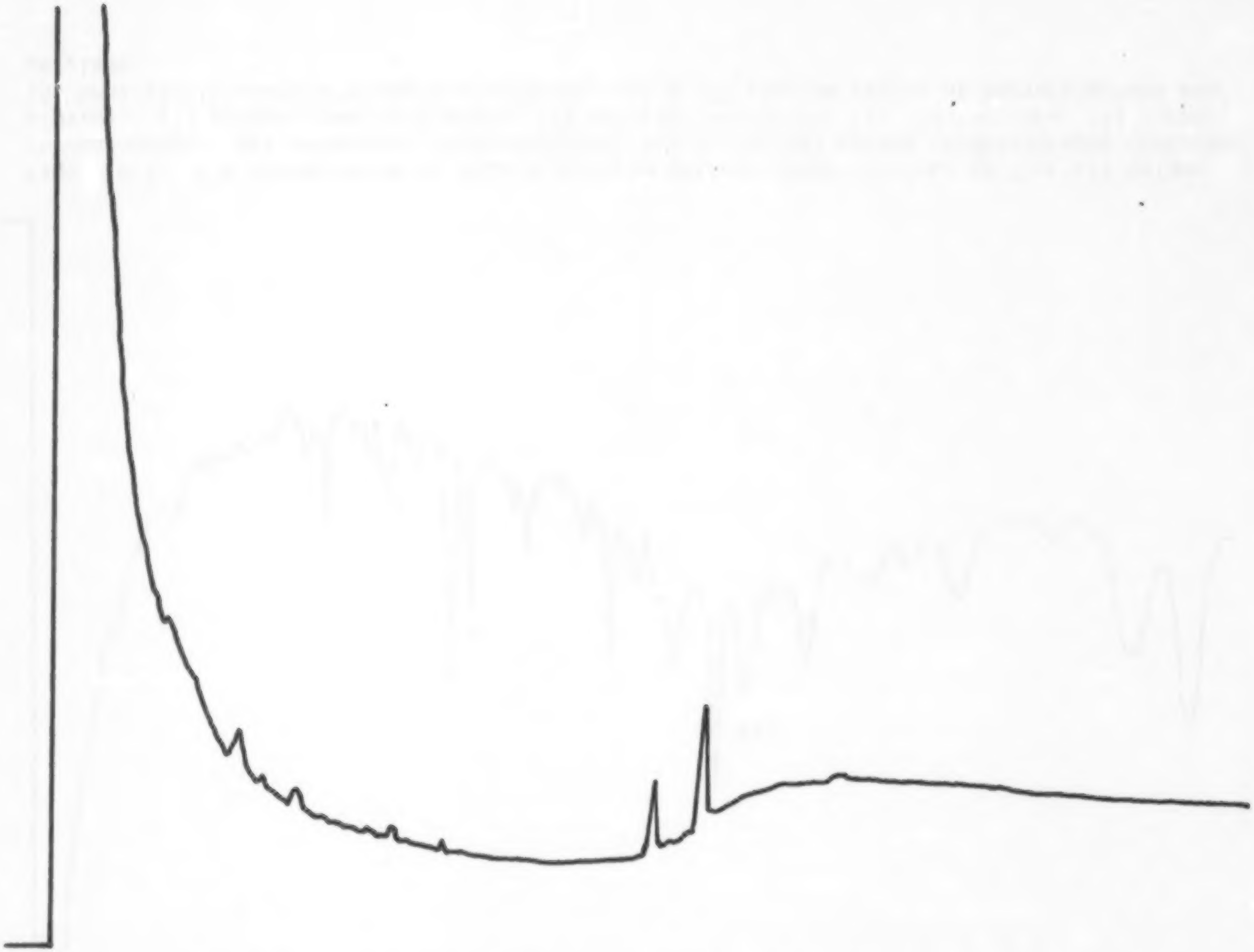


Figure B-1. FID chromatogram of Lake Superior sediment extract after clean-up by florisil column chromatography, gel permeation chromatography, and silica gel column chromatography (benzene eluate).

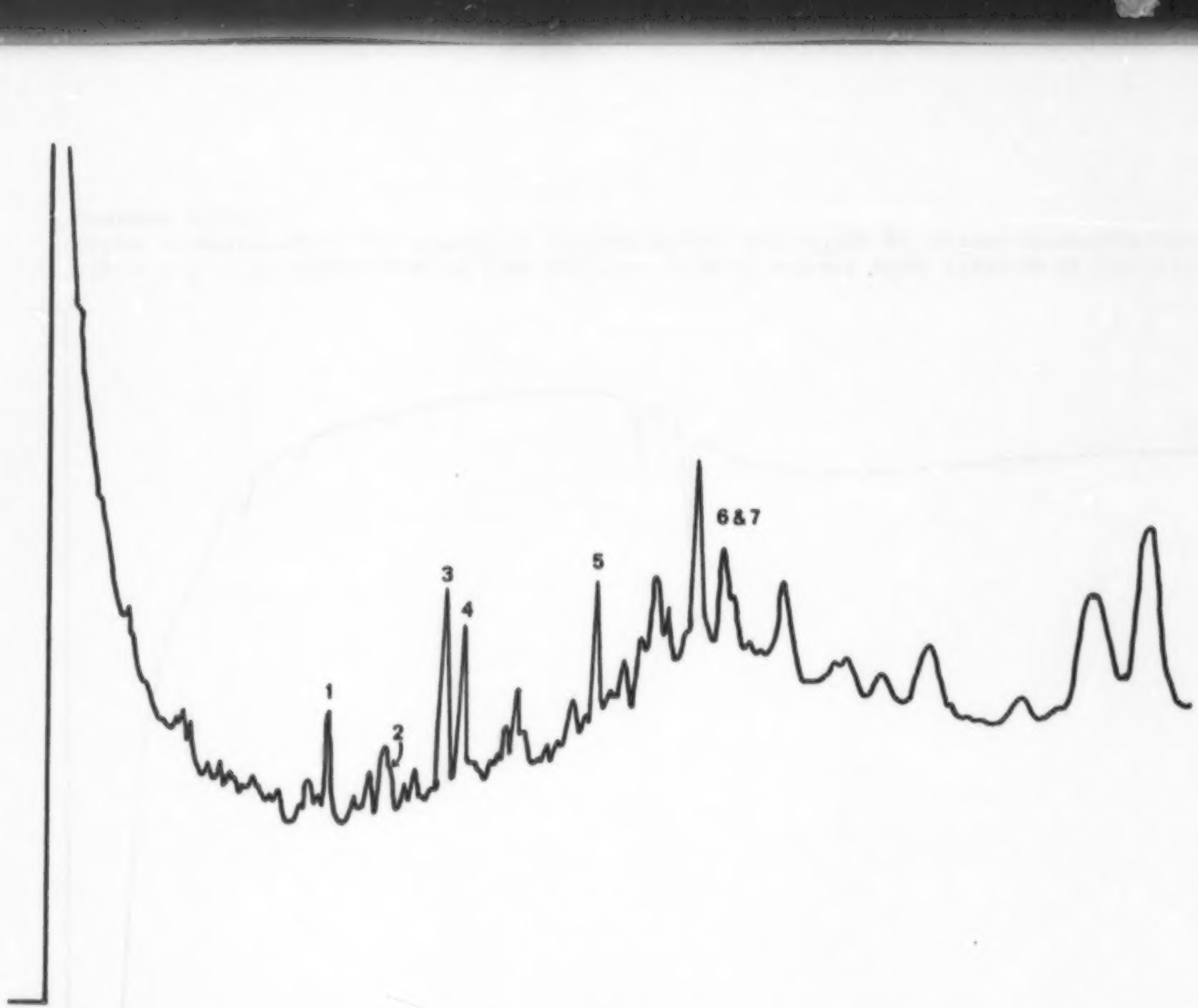


Figure B-2. FID chromatogram of Site 4 sediment extract after clean-up by florisil column chromatography, gel permeation chromatography, and silica gel column chromatography (benzene eluate). (1) phenanthrene/anthracene, (2) methylphenanthrene, (3) fluoranthene, (4) pyrene, (5) benz(a)anthracene/triphenylene/chrysene, (6) & (7) elution region of benzo(a)pyrene and perylene.

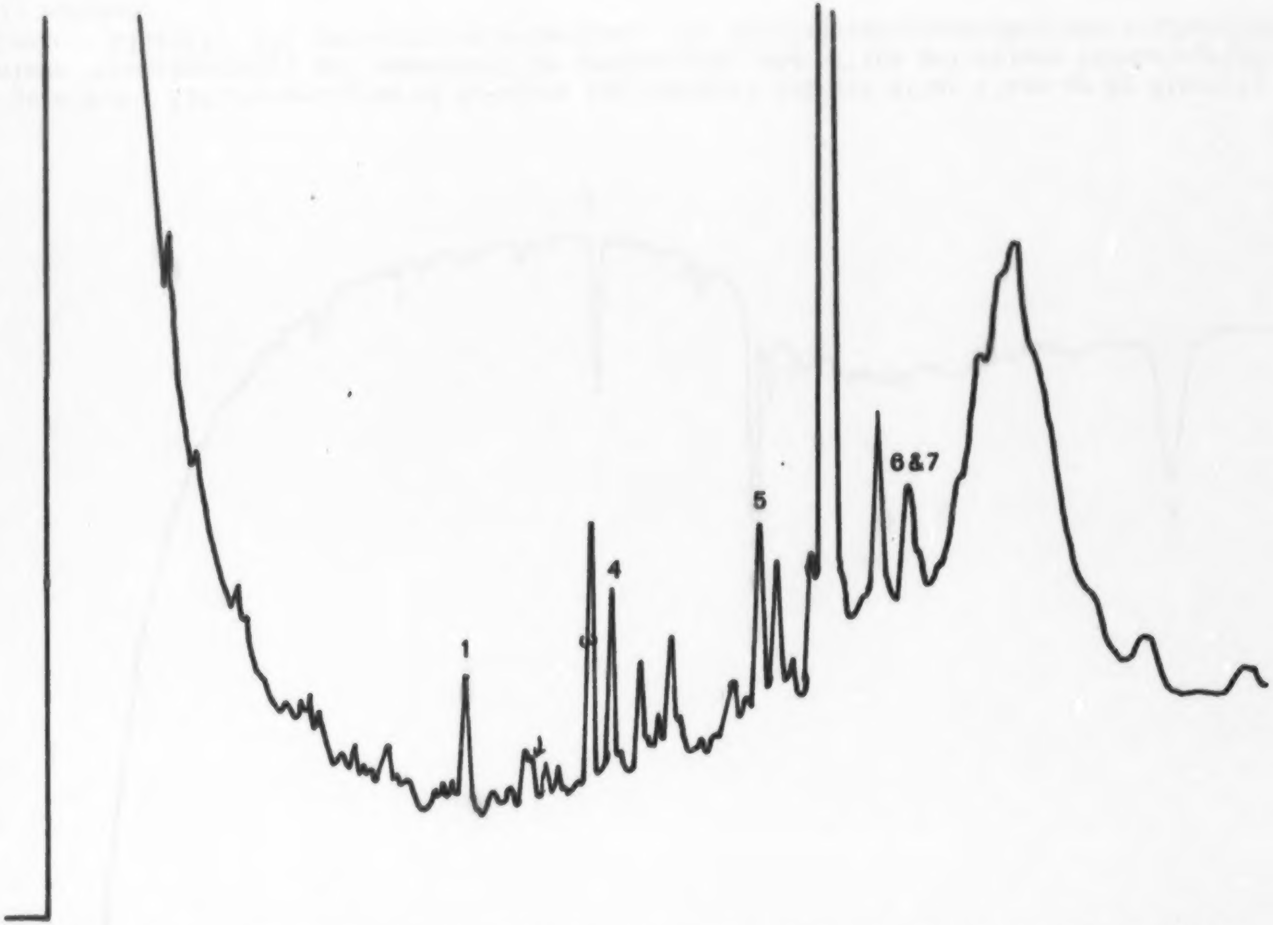


Figure B-3. FID chromatogram of Site 6 sediment extract after clean-up by florisil column chromatography, gel permeation chromatography, and silica gel column chromatography (benzene eluate). (1) phenanthrene/anthracene, (2) methylphenanthrene, (3) fluoranthene, (4) pyrene, (5) benz(a)anthracene/chrysene/triphenylene, (6) & (7) elution region of benzo(a)pyrene and perylene.

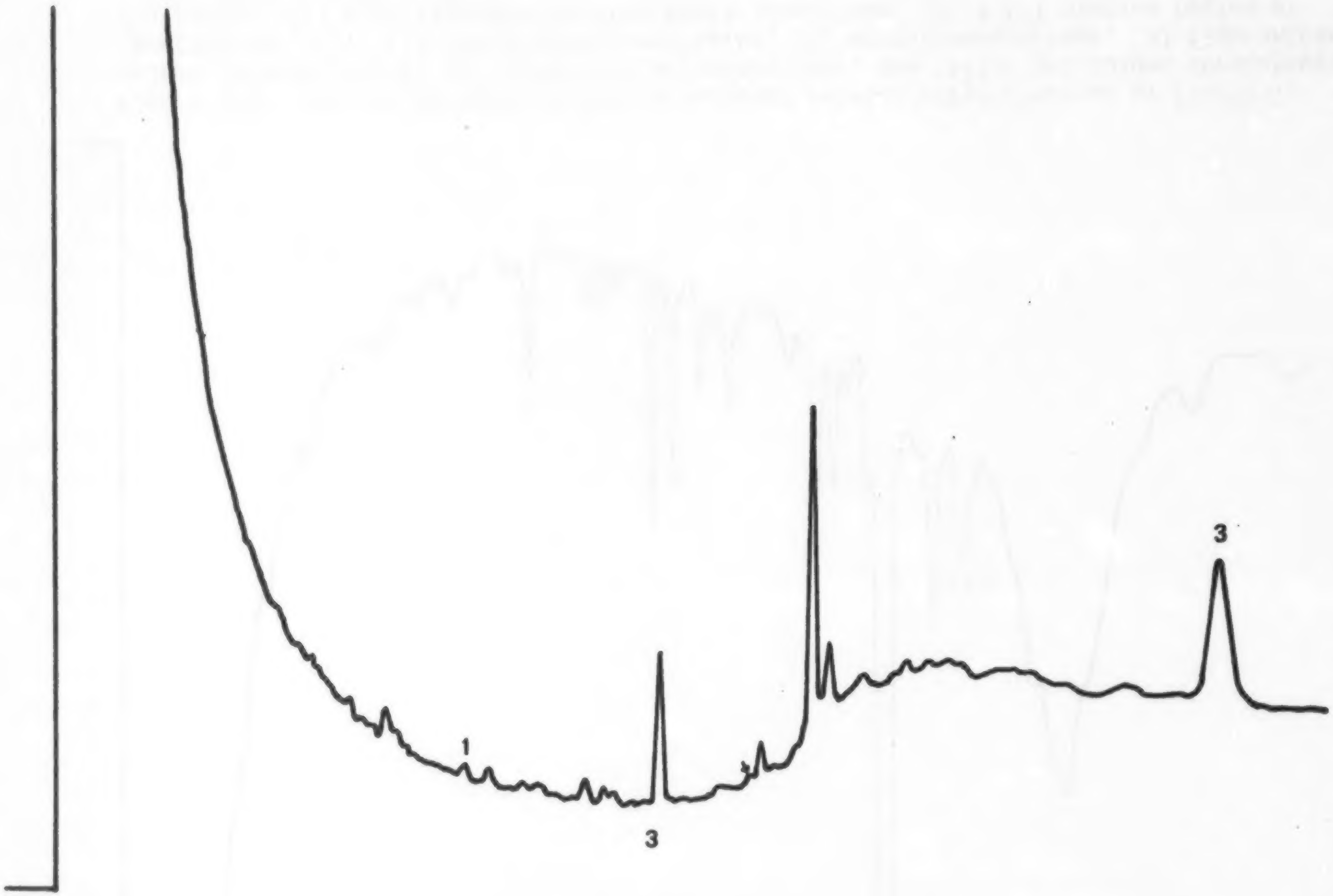


Figure B-4. FID chromatogram of Pokegama Bay sediment extract after clean-up by florisil column chromatography, gel permeation chromatography, and silica gel column chromatography (benzene eluate). (1) phenanthrene/anthracene, (2) benz(a)anthracene/chrysene/triphenylene, (3) unknown.

Figure B-5: Reconstructed Ion and Selected Mass Chromatograms

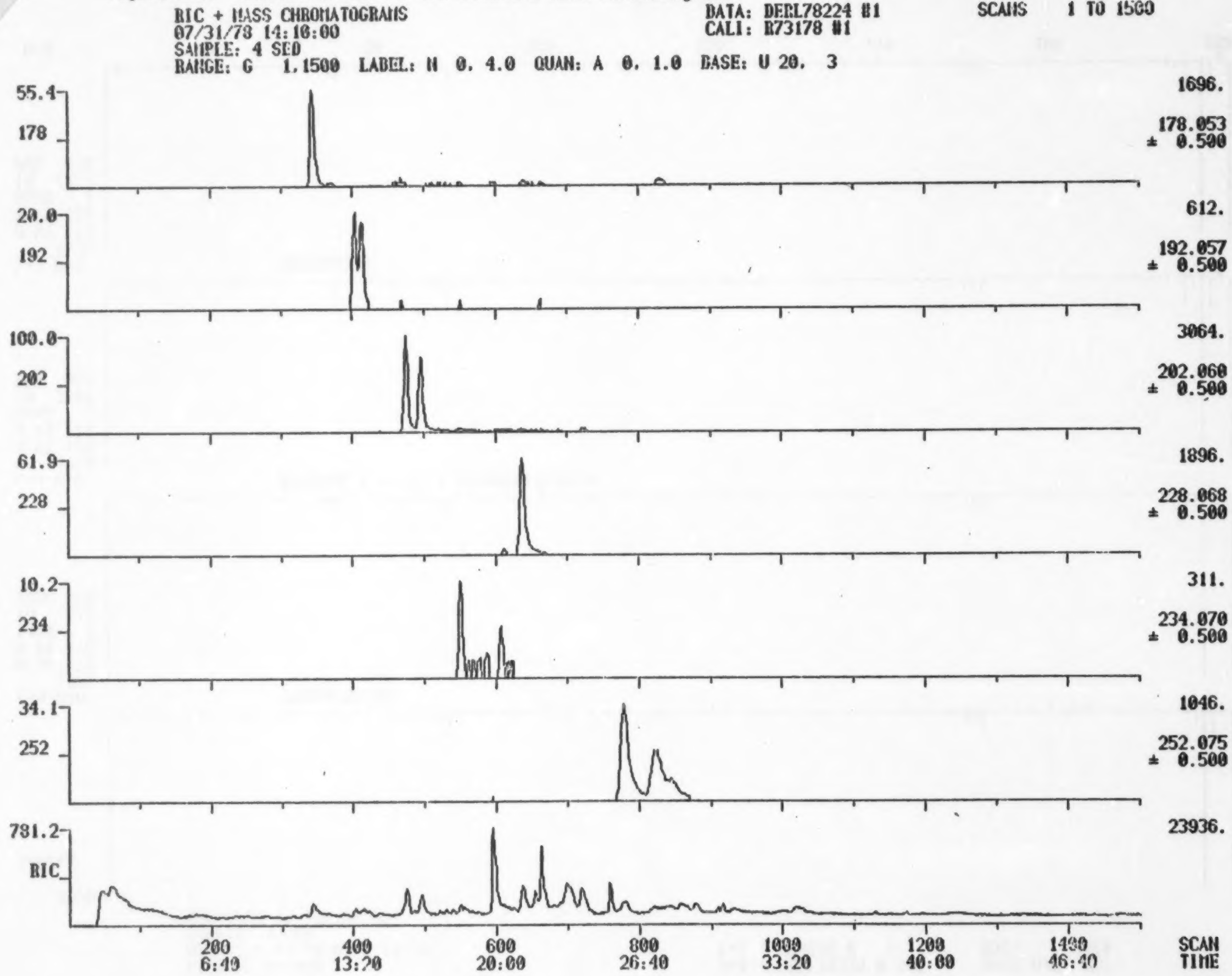


Figure B-6: Mass Spectral Library Search

```
LIBRARY SEARCH                                 DATA: DERL78224 # 345      BASE M/E: 178
07/31/78 14:10:00 + 11:30                      CALI: B73178 #   1         RIC:      3079.
SAMPLE: 4 SED
ENHANCED (S 15B 2N 0T)
```

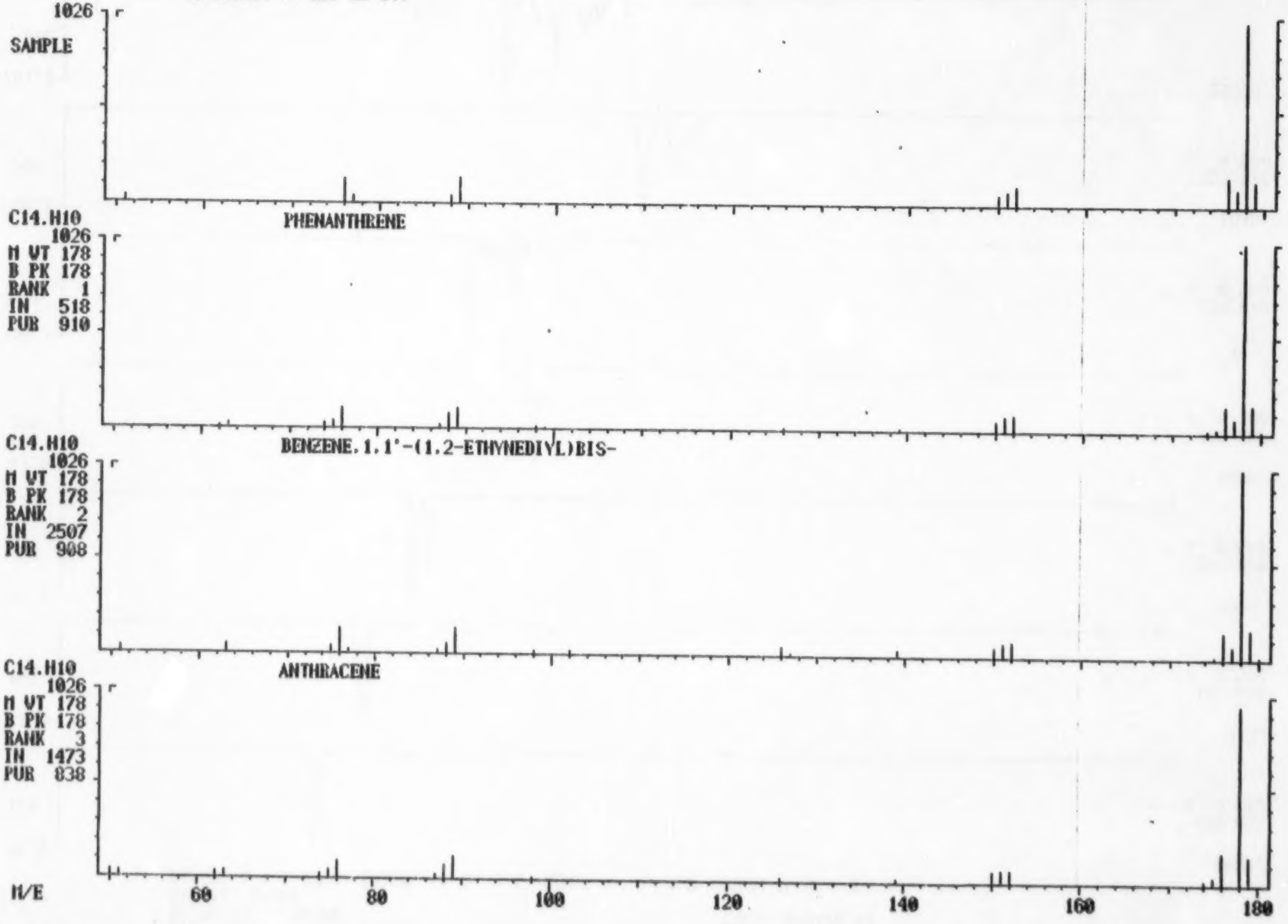


Figure B-6: (continued)

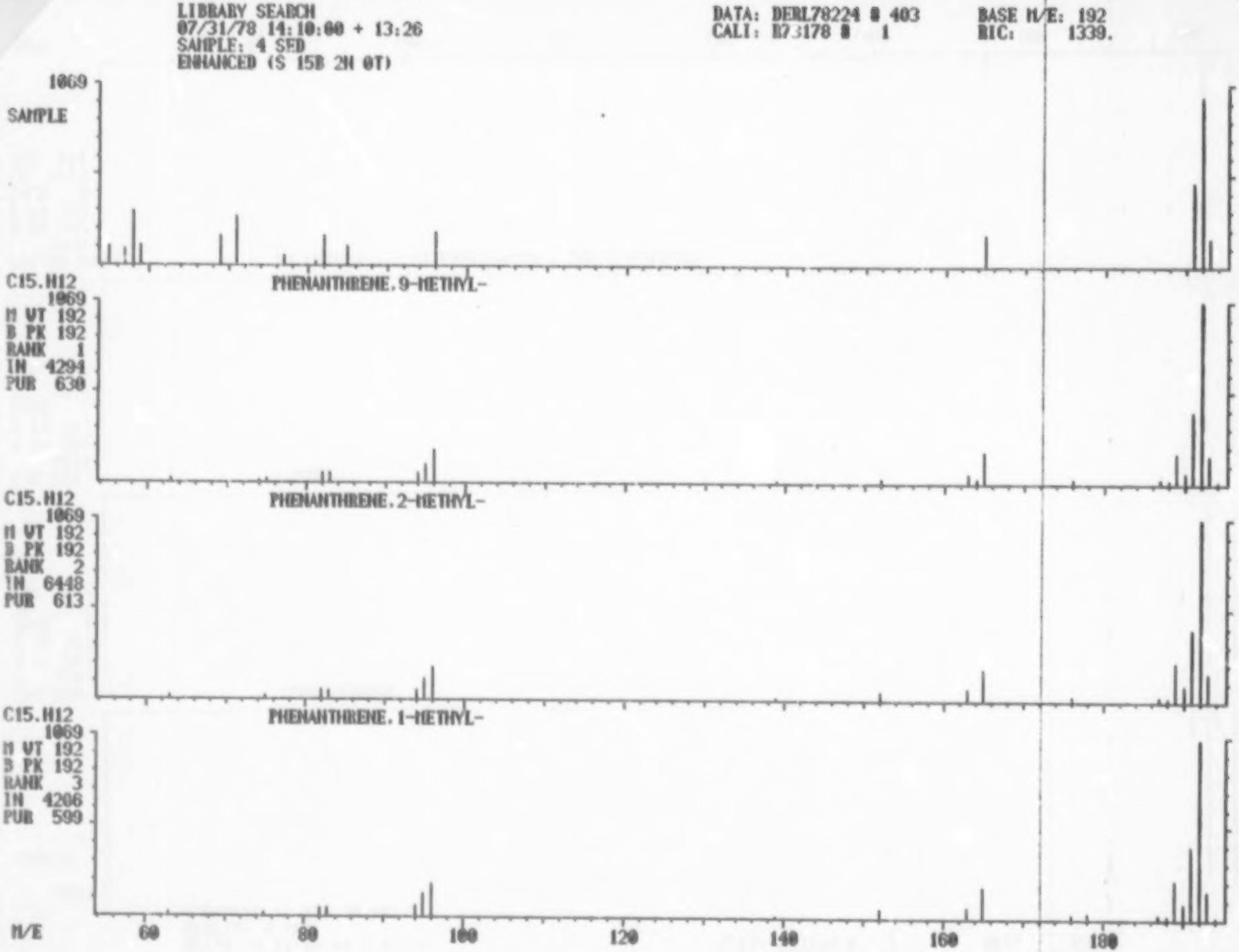


Figure B-6: (continued)
LIBRARY SEARCH
07/31/78 14:10:00 + 15:52
SAMPLE: 4 SED
ENHANCED (S 15B 2N 0T)

DATA: DERL78224 # 476
CALI: R73178 # 1

BASE M/E: 202
RIC: 5319.

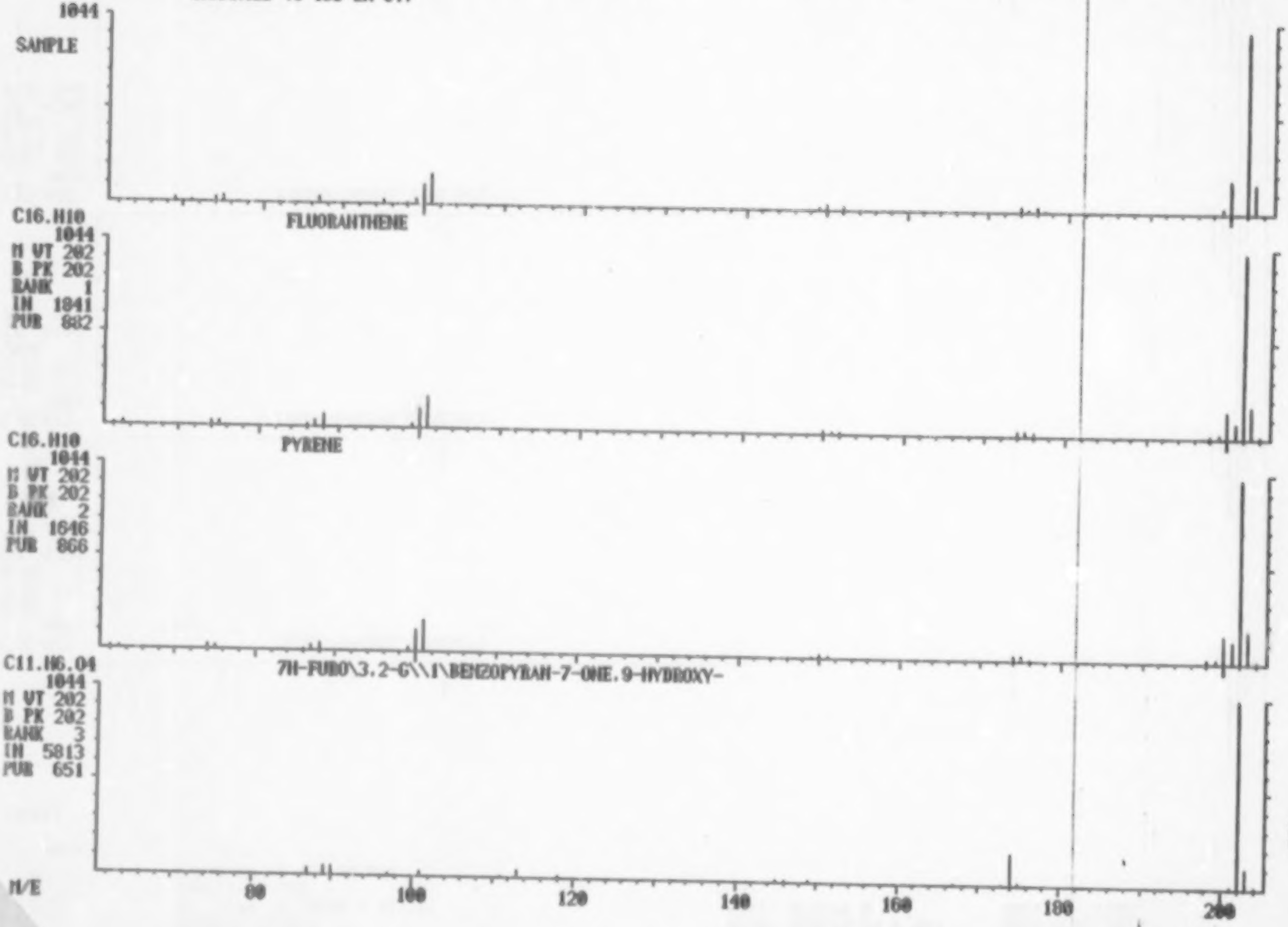


Figure B-6: (continued)

LIBRARY SEARCH
07/31/78 14:10:00 + 16:32
SAMPLE: 4 SED
ENHANCED (S 15B 2N 0T)

DATA: DERL78224 # 496
CALI: B73178 # 1

BASE M/E: 202
RIC: 4439.

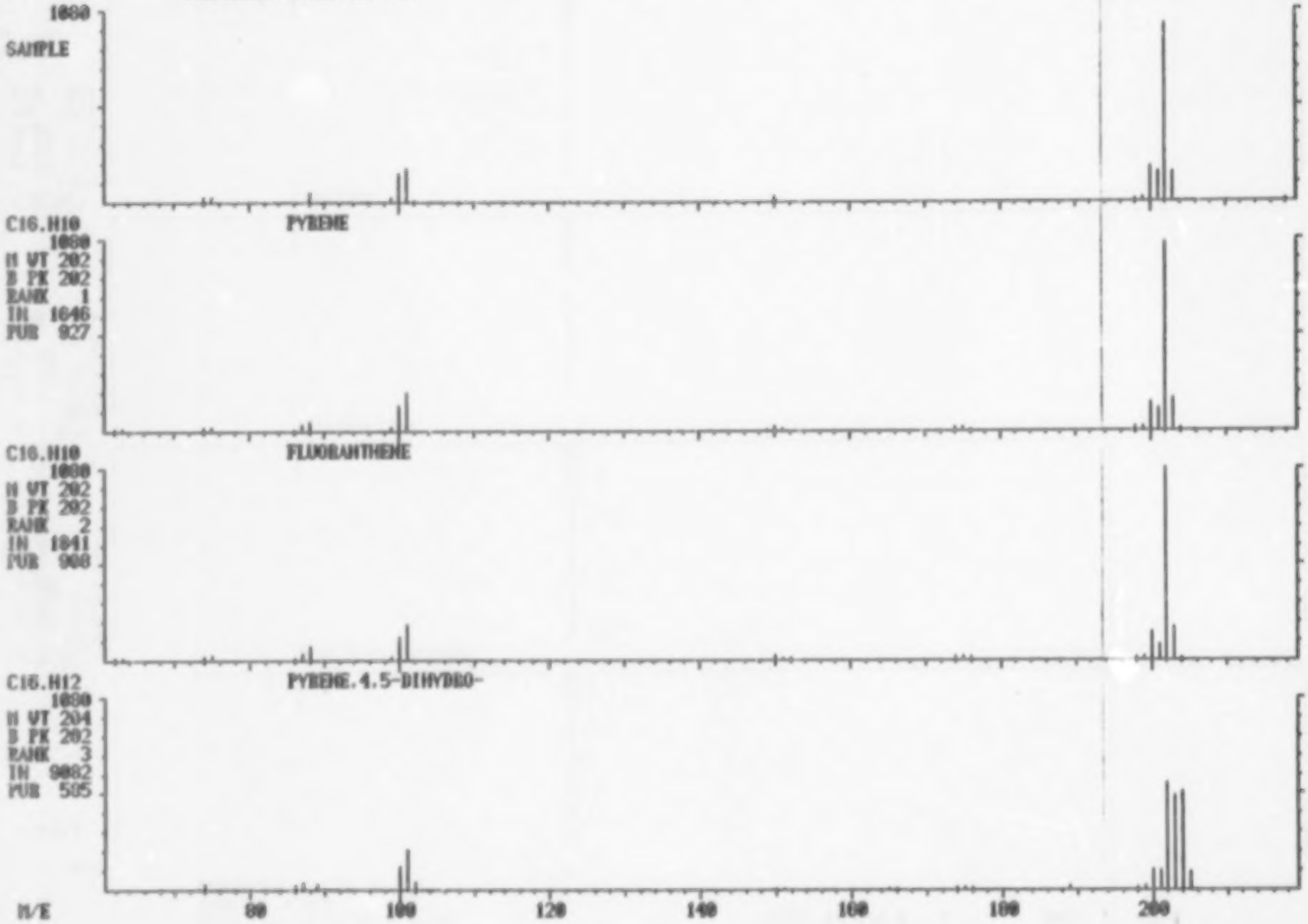


Figure B-6: (continued)
LIBRARY SEARCH
07/31/78 14:10:00 + 21:16
SAMPLE: 4 SED
ENHANCED (S 15B 2N 0T)

DATA: DEBL78224 # 638
CALI: R73178 # 1

BASE M/E: 228
RIC: 4759.

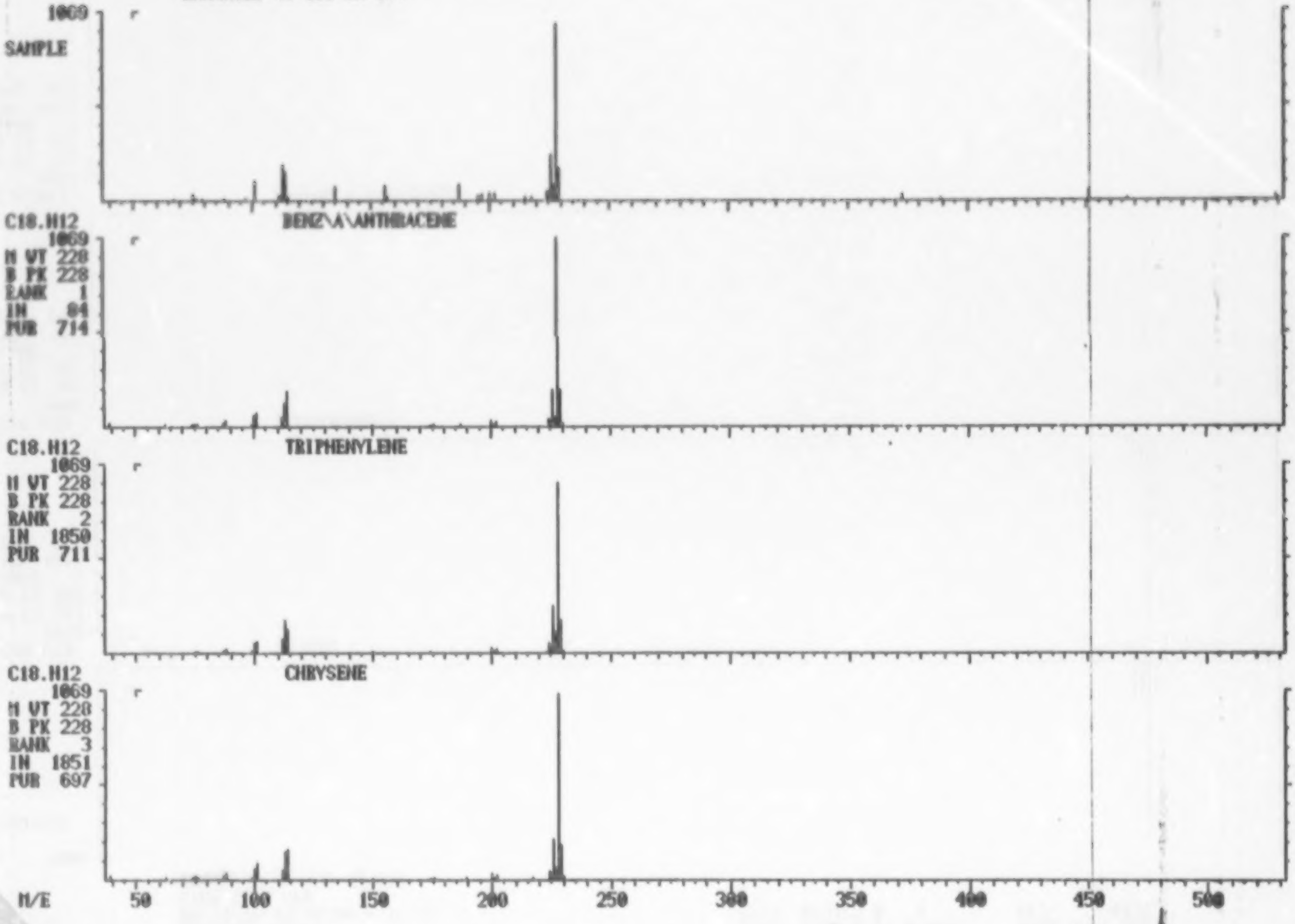


Figure B-6: (continued)

LIBRARY SEARCH
07/31/78 14:10:00 + 18:24
SAMPLE: 4 SED
ENHANCED (S 15B 2N 0T)

DATA: DERL78224 # 552
CALI: R73178 # 1

BASE M/E: 219
RIC: 1931.

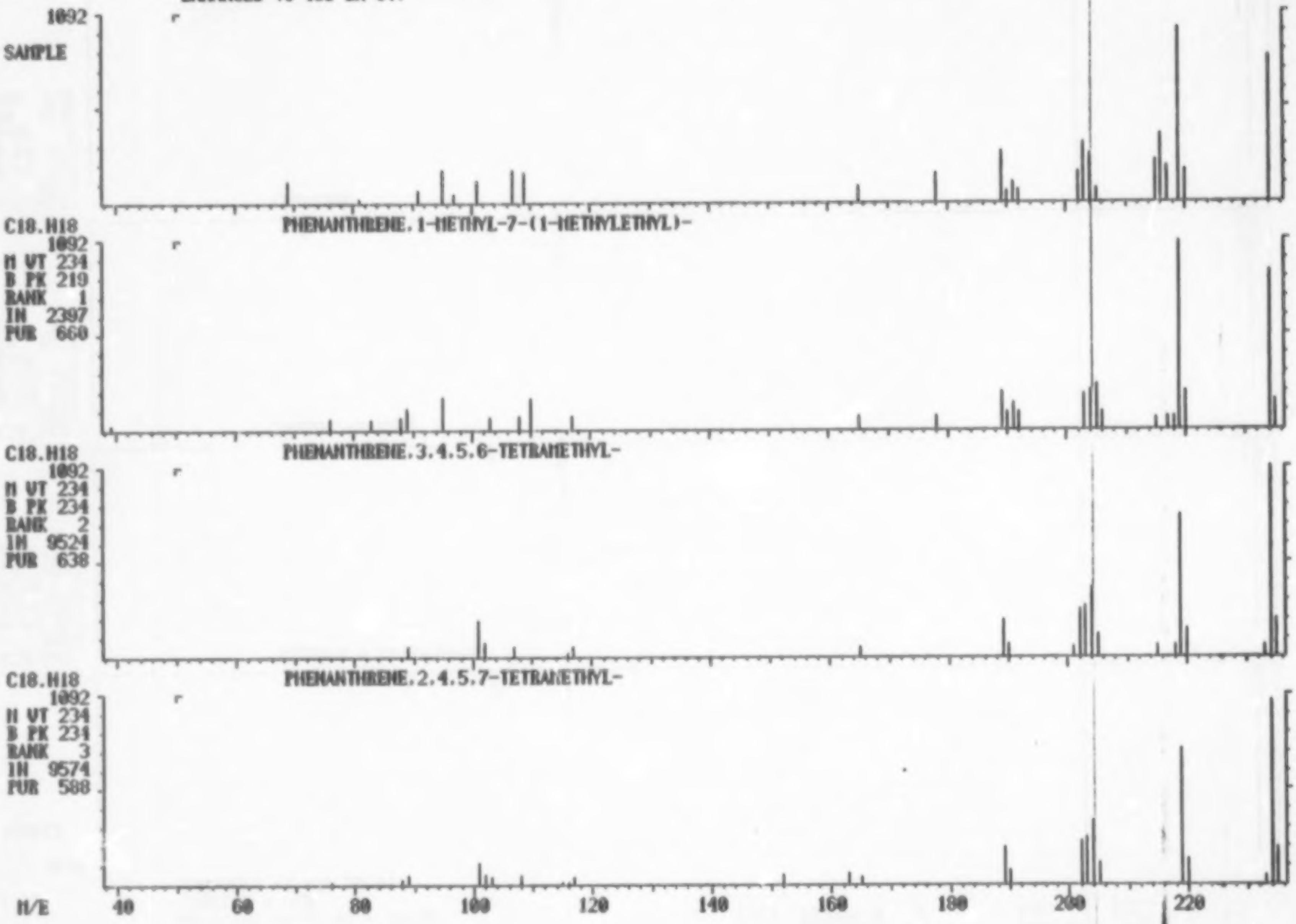


Figure B-6: (continued)

LIBRARY SEARCH
07/31/78 14:10:00 + 26:00
SAMPLE: 4 SED
ENHANCED (S 15B 2N 0T)

DATA: DERL78224 # 780
CALI: R73178 # 1

BASE M/E: 252
RIC: 1643.

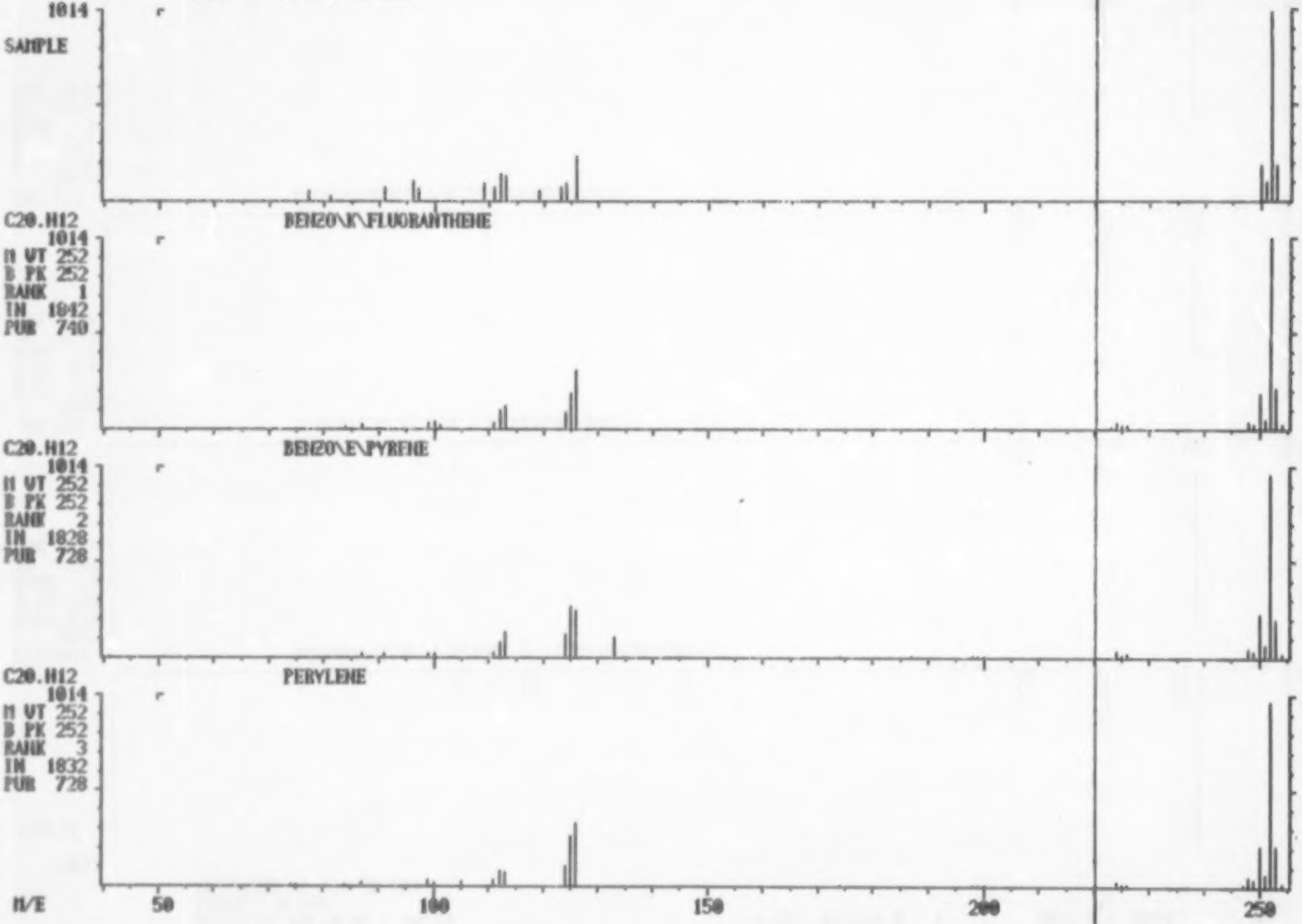
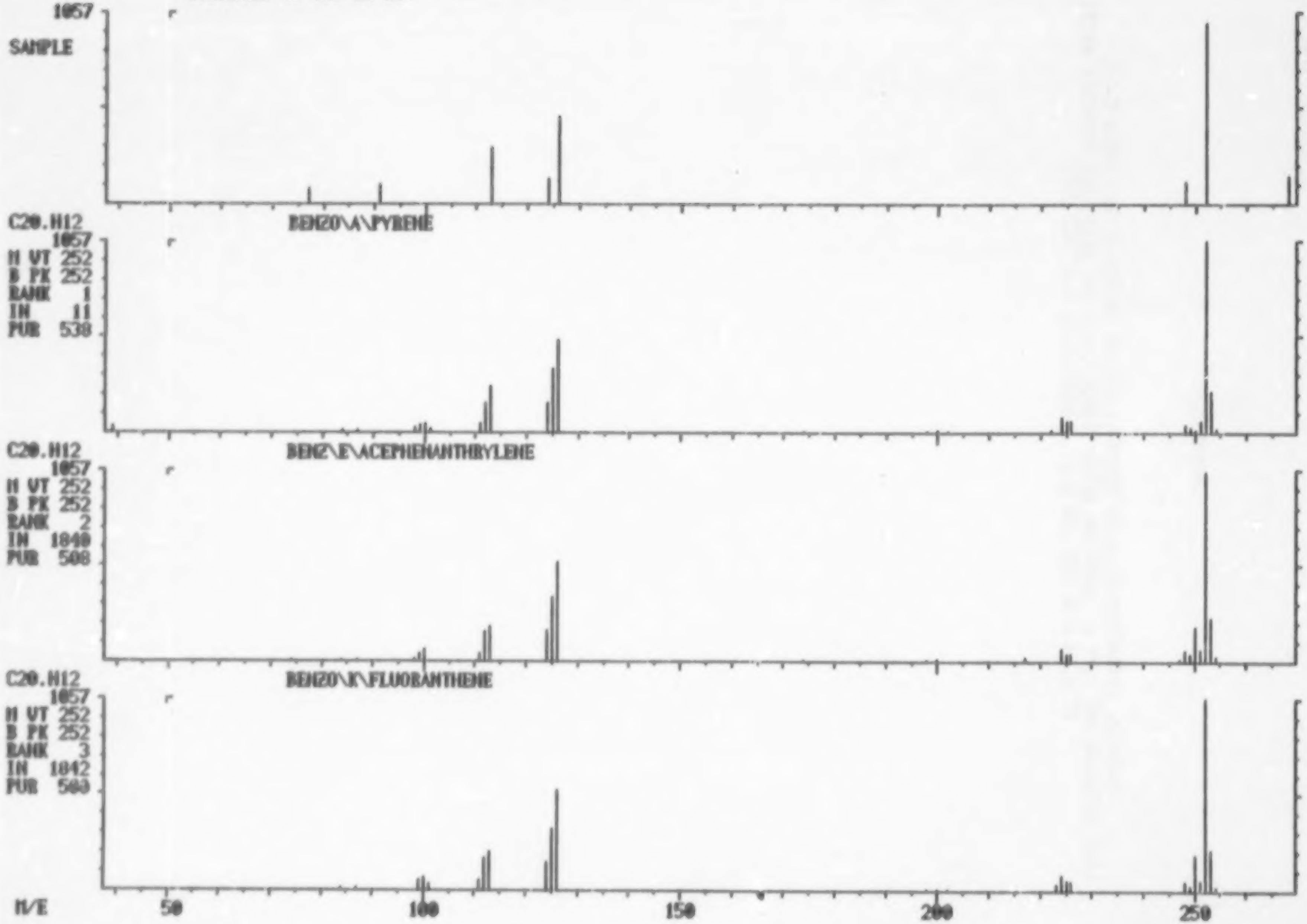


Figure B-6: (continued)

```
LIBRARY SEARCH                                   DATA: DEBL78224 # 826      BASE M/E: 252
07/31/78 14:10:00 + 27:32                        CALI: B73178 #   1         BIC:      377.
SAMPLE: 4 SED
ENHANCED (S 15B 2N 0T)
```



APPENDIX C

CHEMICAL CHARACTERISTICS OF DULUTH-SUPERIOR HARBOR WATER (TABLE C-1), LAKE SUPERIOR AND CITY OF SUPERIOR WATER (TABLE C-2), AND *HEXAGENIA* BIOASSAY WATER AT HIGH D.O. AND LOW D.O. (TABLES C-3, C-4, AND C-5)

CHEMICAL CHARACTERISTICS OF DULUTH-SUPERIOR HARBOR WATER

Measurements of dissolved oxygen (Yellow Springs Model 54A Dissolved Oxygen Meter), specific conductance and temperature (Yellow Springs Model 33 SCT Meter) were made as a function of depth in the overlying water at a number of the Duluth-Superior harbor sampling sites in 1977. Similar measurements were made at the Pokegama Bay reference site. The results of these measurements are given in Table C-1.

TABLE C-1. TEMPERATURE, DISSOLVED OXYGEN AND SPECIFIC CONDUCTANCE PROFILES OF WATER OVERLYING SITE SEDIMENTS*

SITE DESIGNATION AND WATER CHARACTERISTICS**

	1			2		3			4		5A		6		
	June 12, 1977			June 5, 1977		July 10, 1977			July 17, 1977		July 24, 1977		June 26, 1977		
Depth(m)	Temp.	D.O.	S.C.	Temp.	D.O.	Temp.	D.O.	S.C.	Temp.	D.O.	Temp.	D.O.	Temp.	D.O.	S.C.
1	15.0	9.4	126	17.0	9.4	18.0	8.5	140	21.2	5.0	23.1	5.9	23.7	5.7	229
2	15.0	9.3	132	15.5	9.4	18.0	8.5	145	21.2	4.7	23.0	5.4	22.0	5.5	222
3	15.0	9.1	135	15.0	8.6	17.5	8.3	140	20.8	4.5			21.9	4.6	222
4	15.0	9.0	137	14.5	8.4	17.0	8.2	135	20.2	4.3			21.2	4.7	214
5	15.2	8.9	138	14.2	8.2	17.0	8.3	130	19.0	3.9			19.5	4.0	210
6	15.2	8.8	138	13.8	8.6	16.8	8.3	120	18.8	4.1			19.0	3.8	208
7	15.3	8.7	139	13.2	9.0	15.9	8.2	115	18.5	3.9					
8	15.3	8.7	140	12.5	9.6	15.0	7.8	120	18.1	3.7					
9	16.0	8.5	140	12.0	10.0				17.9	2.8					
11															
13															
15															

* Location of sample sites are described in Section 6.

**Water temperature (Temp.) in degrees Celcius, dissolved oxygen (D.O.) in mg/l and specific conductance (S.C.) in micro mhos/cm.

(continued)

Table C-1 (continued)

SITE DESIGNATION AND WATER CHARACTERISTICS

	LS August 7, 1977			6R August 14, 1977		Pokegama June 6, 1977		Pokegama July 17, 1977		Pokegama July 24, 1977	
Depth(m)	Temp.	D.O.	S.C.	Temp.	D.O.	Temp.	D.O.	Temp.	D.O.	Temp.	D.O.
1	18.2	9.7	82	19.0	7.7	22.0	9.8	26.5	6.6	26.0	3.0
2	18.0	---	83	18.0	7.7						
3	17.5	9.9	83	18.0	7.7						
4	17.5	---	83	17.5	7.6						
5	17.2	10.4	83	17.0	7.1						
6	---	---	---	17.0	6.0						
7	16.4	10.0	82								
8	---	---	---								
9	15.4	10.4	81								
11	12.9	10.2	78								
13	11.5	10.4	78								
15	9.8	---	74								

TABLE C-2. COMPARISON OF LAKE SUPERIOR WATER WITH CITY OF SUPERIOR WATER

	Lake Superior Water*			Dechlorinated City of Superior Water
Parameter**	Mean	Range	No. Samples	Mean†
pH	7.74	7.4-8.2	23	7.1
Total Hardness	45,300	44,000-53,000	54	49,100
Alkalinity	42,300	41,000-50,000	54	49,000
Chloride	1,217	1,170-1,340	18	4,100
Sodium	1,130	1,090-1,190	23	1,800
Calcium	13,695	13,000-14,700	23	14,600
Magnesium	3,123	2,940-3,590	23	3,300
Potassium	534	480-590	23	520
Iron	23	2-83	10	194
Manganese	---	0.2-11.5	23	7.8
Chromium	---	2-20	4	<0.1
Aluminum	---	1-26	5	6
Zinc	0.78	1-2.7	21	3.0
Nickel	<0.5	---	23	1.3
Lead	---	7-20	2	<0.1
Copper	1.51	0.3-3.2	23	1.3
Cobalt	<0.5	---	23	<0.5
Mercury	<0.01	---	5	---
Cadmium	<0.1	---	23	<0.05

* Data from Biesinger and Christensen (1972).

**Values in µg/l except for pH.

† Average of three samples.

TABLE C-3. TEMPERATURE, D.O. AND TURBIDITY CONDITIONS DURING OVERSEDIMENT BIOASSAYS*

Site	Temperature**(°C)				Dissolved Oxygen**(mg/l)				Turbidity**			
	Test Site		Control		Test Site		Control		Test Site		Control	
	Low O_2	High O_2	Low O_2	High O_2	Low O_2	High O_2	Low O_2	High O_2	Low O_2	High O_2	Low O_2	High O_2
1	18.8 (0.5)	18.5 (0.6)	18.7 (0.5)	18.5 (0.6)	4.5 (1.1)	8.2 (0.3)	4.2 (1.1)	8.2 (0.4)	50 (45)	71 (58)	112 (97)	155 (122)
2	18.9 (0.5)	18.6 (0.5)	18.9 (0.5)	18.6 (0.5)	4.4 (1.3)	8.0 (0.4)	4.1 (1.3)	7.9 (1.3)	526 (111)	723 (212)	273 (85)	571 (190)
3	18.5 (0.3)	18.3 (0.2)	18.4 (0.3)	18.2 (0.2)	4.0 (0.3)	8.0 (0.4)	4.4 (1.1)	8.2 (0.4)	180 (52)	241 (53)	171 (55)	234 (56)
4	18.7 (0.4)	18.5 (0.3)	18.6 (0.4)	18.5 (0.3)	3.6 (1.1)	7.7 (0.9)	3.8 (1.2)	7.6 (1.1)	195 (67)	291 (69)	274 (83)	351 (119)
5a	18.9 (0.4)	18.7 (0.4)	18.8 (0.5)	18.6 (0.4)	4.2 (1.4)	7.3 (0.4)	4.1 (1.4)	7.1 (0.5)	116 (33)	228 (89)	265 (95)	440 (179)
6	19.0 (0.5)	18.5 (0.4)	18.9 (0.5)	18.5 (0.4)	4.0 (1.2)	8.0 (0.3)	4.2 (1.1)	8.1 (0.3)	78 (26)	169 (82)	144 (60)	298 (91)
LS	18.3 (0.3)	18.2 (0.3)	18.3 (0.3)	18.2 (0.3)	4.9 (1.4)	8.8 (0.1)	4.6 (1.6)	8.8 (0.2)	171 (59)	327 (124)	265 (74)	468 (163)
6R	18.6 (0.3)	18.5 (0.3)	18.6 (0.3)	18.5 (0.3)	3.9 (1.3)	8.4 (0.8)	4.5 (1.3)	8.6 (0.3)	151 (44)	226 (55)	277 (80)	414 (164)

* See Section 8 for procedural description.

** The number in parentheses are standard deviations. Turbidity is given in Formazin Turbidity Units (FTUs).

TABLE C-4. HEXAGENIA POST-BIOASSAY WATER (HIGH D.O.)

	SITE								
Parameter*	1	2	3	4	5a	6	6R	LS	Pokegema
pH	7.9	7.3	7.1	7.4	7.5	---	7.4	7.5	---
Total Phenols	0.015	0.032	0.033	0.027	0.027	0.005	0.042	0.013	0.023
H_2S	<0.17	<0.17	0.20	0.20	<0.17	<0.17	<0.17	0.19	<0.17
Ammonia	0.74	2.9	7.5	7.9	2.5	1.7	7.9	5.7	2.6
TKN	4.6	2.9	10.5	16	12	7.2	16	11	7.4
COD	10.3	23.5	17.4	17.0	20.1	5.8	3.9	11.3	21.9
Organic N	3.9	0	3.0	8.1	9.5	5.5	8.1	5.3	4.8
Arsenic	4.1	1.1	3.3	2.6	3.6	3.9	3.9	2.9	3.0
Cadmium	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Chromium	1.5	1.1	0.7	0.9	2.2	1.3	1.3	3.6	0.9
Cobalt	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Copper	13	10	13	10	14	6	10	29	12
Iron	360	570	630	760	630	360	510	510	360
Lead	0.6	0.7	1.4	1.5	1.7	1.0	0.8	0.8	0.8
Manganese	230	350	115	180	90	130	130	15	27
Nickel	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	2.1	2.1	<2.0
Selenium	4	1.5	1	1	1	4	1.5	1	4
Zinc	2.2	4.6	5.5	7.4	5.0	3.2	37	36	4.6

*Values in mg/l except for metals which are given in μg/l.

TABLE C-5. HEXAGENIA POST-BIOASSAY WATER (LOW D.O.)

	SITE								
Parameter*	1	2	3	4	5a	6	6R	LS	Pokegema
pH	7.5	7.1	7.3	6.9	7.3	---	7.2	7.5	---
Total Phenols	0.014	---	0.011	0.025	0.034	0.016	0.028	0.016	0.020
H_2S	<0.17	<0.17	---	<0.17	<0.17	<0.17	<0.17	0.19	<0.17
Ammonia	1.8	1.7	9.3	8.9	4.6	5.5	7.3	5.3	4.3
TKN	3.9	1.7	16	13	13	10	13	12	9
COD	14.0	23.3	18.7	23.5	16.0	6.6	6.3	7.9	2.5
Organic N	2.1	0	6.7	4.1	8.4	4.5	5.7	6.7	4.7
Arsenic	2.6	4.5	3.1	3.6	3.6	5.6	4.6	2.4	2.1
Cadmium	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	0.05
Chromium	0.5	0.8	0.5	0.6	0.7	4.0	0.8	2.3	1.5
Cobalt	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Copper	21	20	9	14	14	10	4	32	17
Iron	210	640	700	760	630	1100	250	510	430
Lead	0.3	0.6	2.1	1.7	1.3	3.2	0.7	1.9	0.8
Manganese	550	610	320	295	110	355	275	19	210
Nickel	<2	<2	<2	<2	<2	<2	2.1	<2	<2
Selenium	4	4	4	4	1	1	4	4	1
Zinc	1.6	3.2	3.5	10	5.0	5.3	5.0	5.9	4.0

*Values in mg/l except for metals which are given in μg/l.

APPENDIX D

GREAT LAKES HARBOR SEDIMENT EVALUATION CRITERIA (EPA)

GREAT LAKES HARBOR SEDIMENT EVALUATION CRITERIA (EPA)*

Guidelines for the evaluation of Great Lakes harbor sediments, based on bulk sediment analysis, have been developed by Region V of the U.S. Environmental Protection Agency. These guidelines, developed under the pressure of the need to make immediate decisions regarding the disposal of dredged material, have not been adequately related to the impact of the sediments on the lakes and are considered interim guidelines until more scientifically sound guidelines are developed.

The guidelines are based on the following facts and assumptions:

1. Sediments that have been severely altered by the activities of man are most likely to have adverse environmental impacts.
2. The variability of the sampling and analytical techniques is such that the assessment of any sample must be based on all factors and not on any single parameter with the exception of mercury and polychlorinated biphenyls (PCB's).
3. Due to the documented bioaccumulation of mercury and PCB's, rigid limitations are used which override all other considerations.

Sediments are classified as heavily polluted, moderately polluted, or nonpolluted by evaluating each parameter measured against the scales shown below. The overall classification of the sample is based on the most predominant classification of the individual parameters. Additional factors such as elutriate test results, source of contamination, particle size distribution, benthic macroinvertebrate populations, color and odor are also considered. These factors are interrelated in a complex manner and their interpretation is necessarily somewhat subjective.

---

*This material is reproduced from the following report:
Guidelines for the Pollution Classification of Great Lakes Harbor Sediments, U.S. Environmental Protection Agency, Region V, April, 1977.

Following ranges used to classify sediments from Great Lakes harbors are based on compilations of data from over 100 different harbors since 1967.

	NONPOLLUTED	MODERATELY POLLUTED	HEAVILY POLLUTED
Volatile Solids (%)	<5	5 - 8	>8
COD (mg/kg dry weight)	<40,000	40,000-80,000	>80,000
TKN (mg/kg dry weight)	< 1,000	1,000-2,000	> 2,000
Oil and Grease (Hexane Solubles) (mg/kg dry weight)	< 1,000	1,000-2,000	> 2,000
Lead (mg/kg dry weight)	< 40	40-60	> 60
Zinc (mg/kg dry weight)	< 90	90-200	> 200

The following supplementary ranges used to classify sediments from Great Lakes harbors have been developed to the point where they are usable but are still subject to modification by the addition of new data. These ranges are based on 260 samples from 34 harbors sampled during 1974 and 1975.

	NONPOLLUTED	MODERATELY POLLUTED	HEAVILY POLLUTED
Ammonia (mg/kg dry weight)	<75	75-200	>200
Cyanide " " "	<0.10	0.10-0.25	>0.25
Phosphorus " " "	<420	420-650	>650
Iron " " "	<17,000	17,000-25,000	>25,000
Nickel " " "	<20	20-50	>50
Manganese " " "	<300	300-500	>500
Arsenic " " "	<3	3-8	>8
Cadmium " " "	*	*	>6
Chromium " " "	<25	25-75	>75
Barium " " "	<20	20-60	>60
Copper " " "	<25	25-50	>50

*Lower limits not established

The guidelines stated below for mercury and PCB's are based upon the best available information and are subject to revision as new information becomes available.

Methylation of mercury at levels ≥ 1 mg/kg has been documented (1,2). Methyl mercury is directly available for bioaccumulation in the food chain.

Elevated PCB levels in large fish have been found in all of the Great Lakes. The accumulation pathways are not well understood. However, bioaccumulation of PCB's at levels ≥ 10 mg/kg in fathead minnows has been documented (3).

Because of the known bioaccumulation of these toxic compounds, a rigid limitation is used. If the guideline values are exceeded, the sediments are classified as polluted and unacceptable for open lake disposal no matter what the other data indicate.

	POLLUTED
Mercury	≥ 1 mg/kg dry weight
Total PCB's	≥ 10 mg/kg dry weight

The pollutional classification of sediments with total PCB concentrations between 1.0 mg/kg and 10.0 mg/kg dry weight will be determined on a case-by-case basis.

a. Elutriate test results.

The elutriate test was designed to simulate the dredging and disposal process. In the test, sediment and dredging site water are mixed in the ratio of 1:4 by volume. The mixture is shaken for 30 minutes, allowed to settle for 1 hour, centrifuged, and filtered through a 0.45 μ filter. The filtered water (elutriate water) is then chemically analyzed.

A sample of the dredging site water used in the elutriate test is filtered through a 0.45 μ filter and chemically analyzed.

A comparison of the elutriate water with the filtered dredging site water for like constituents indicates whether a constituent was or was not released in the test.

The value of elutriate test results are limited for overall pollutional classification because they reflect only immediate release to the water column under aerobic and near neutral pH conditions. However, elutriate test results can be used to confirm releases of toxic materials and to influence decisions where bulk sediment results are marginal between two classifications. If there is release or non-release, particularly of a more toxic constituent, the elutriate test results can shift the classification toward the more polluted or the less polluted range, respectively.

b. Source of sediment contamination.

In many cases the sources of sediment contamination are readily apparent. Sediments reflect the inputs of paper mills, steel mills, sewage discharges and heavy industry very faithfully. Many sediments may have moderate or high concentrations of TKN, COD, and volatile solids yet exhibit no evidence of man made pollution. This usually occurs when drainage from a swampy area reaches the channel or harbor, or when the project itself is located in a low lying wetland area. Pollution in these projects may be considered natural and some leeway may be given in the range values for TKN, COD, and volatile solids provided that toxic materials are not also present.

c. Field observations.

Experience has shown that field observations are a most reliable indicator of sediment condition. Important factors are color, texture, odor, presence of detritus, and presence of oily material.

Color. A general guideline is the lighter the color the cleaner the sediment. There are exceptions to this rule when natural deposits have a darker color. These conditions are usually apparent to the sediment sampler during the survey.

Texture. A general rule is the finer the material the more polluted it is. Sands and gravels usually have low concentrations of pollutants while silts usually have higher concentrations. Silts are frequently carried from polluted upstream areas, whereas, sand usually comes from lateral drift along the shore of the lake. Once again, this general rule can have exceptions and it must be applied with care.

Odor. This is the odor noted by the sampler when the sample is collected. These odors can vary widely with temperature and observer and must be used carefully. Lack of odor, a beach odor, or a fishy odor tends to denote cleaner samples.

Detritus. Detritus may cause higher values for the organic parameters COD, TKN, and volatile solids. It usually denotes pollution from natural sources. Note: The determination of the "naturalness" of a sediment depends upon the establishment of a natural organic source and a lack of man made pollution sources with low values for metals and oil and grease. The presence of detritus is not decisive in itself.

Oily material. This almost always comes from industry or shipping activities. Samples showing visible oil are usually highly contaminated. If chemical results are marginal, a notation of oil is grounds for declaring the sediment to be polluted.

d. Benthos.

Classical biological evaluation of benthos is not applicable to harbor or channel sediments because these areas very seldom support a well balanced population. Very high concentrations of tolerant organisms indicate organic contamination but do not necessarily preclude open lake disposal of the sediments. A moderate concentration of oligochaetes or other tolerant organisms frequently characterizes an acceptable sample. The worst case exists when there is a complete lack or very limited number of organisms. This may indicate a toxic condition.

In addition, biological results must be interpreted in light of the habitat provided in the harbor or channel. Drifting sand can be a very harsh habitat which may support only a few organisms. Silty material, on the other hand, usually provides a good habitat for sludgeworms, leeches, fingernail clams, and perhaps, amphipods. Material that is frequently disturbed by ship's propellers provides a poor habitat.

REFERENCES

1. Jensen, S., and Jernelöv, A., "Biological Methylation of Mercury in Aquatic Organisms," Nature, 223, August 16, 1969 pp 753-754.

2. Magnuson, J.J., Forbes, A., and Hall, R., "Final Report - An Assessment of the Environmental Effects of Dredged Material Disposal in Lake Superior - Volume 3: Biological Studies," Marine Studies Center, University of Wisconsin, Madison, March, 1976.

3. Halter, M.T., and Johnson, H.E., "A Model System to Study the Release of PCB from Hydrosoils and Subsequent Accumulation by Fish," presented to American Society for Testing and Materials, Symposium on Aquatic Toxicology and Hazard Evaluation," October 25-26, 1976, Memphis, Tennessee.